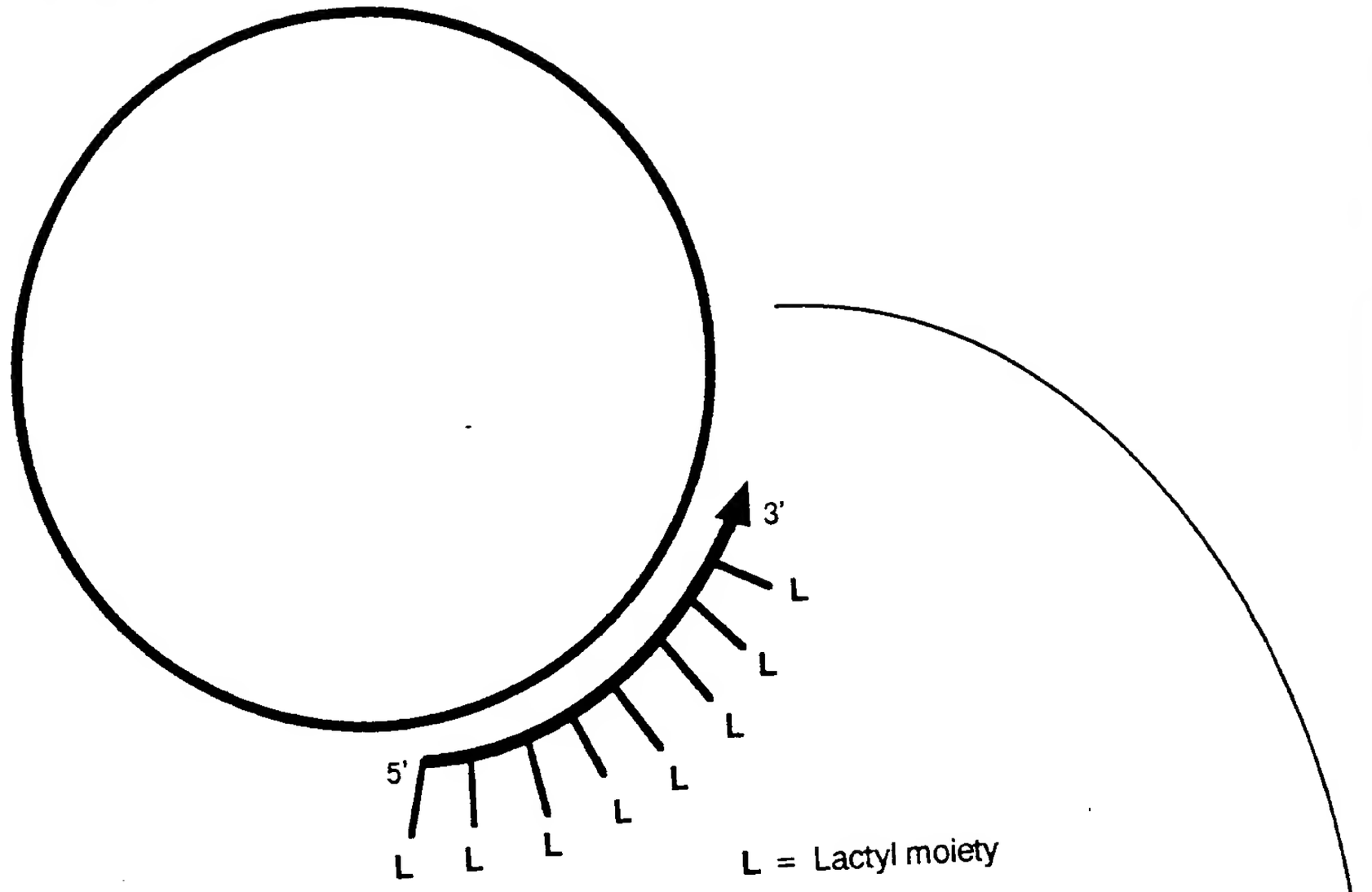
(a)



(b)

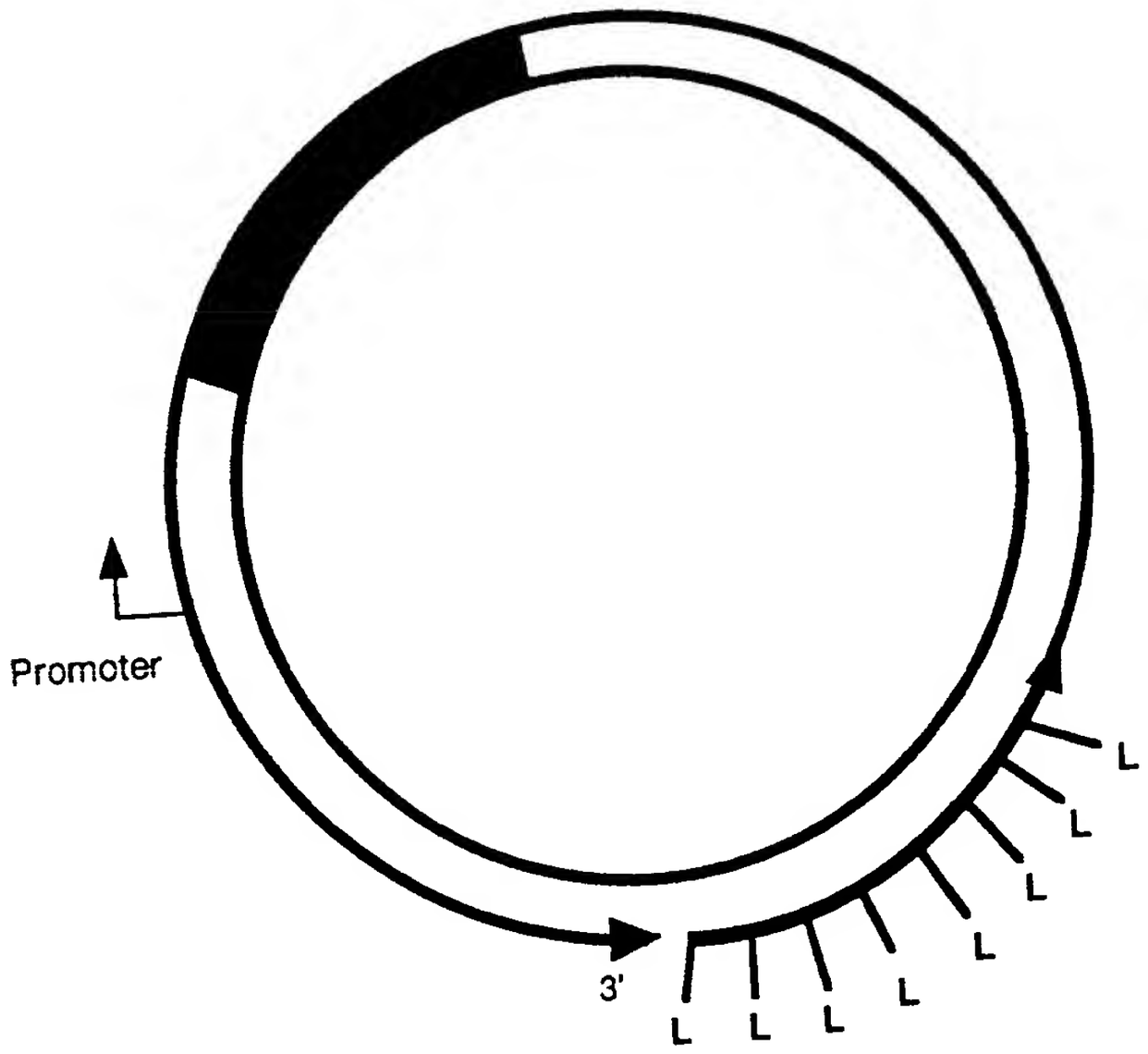
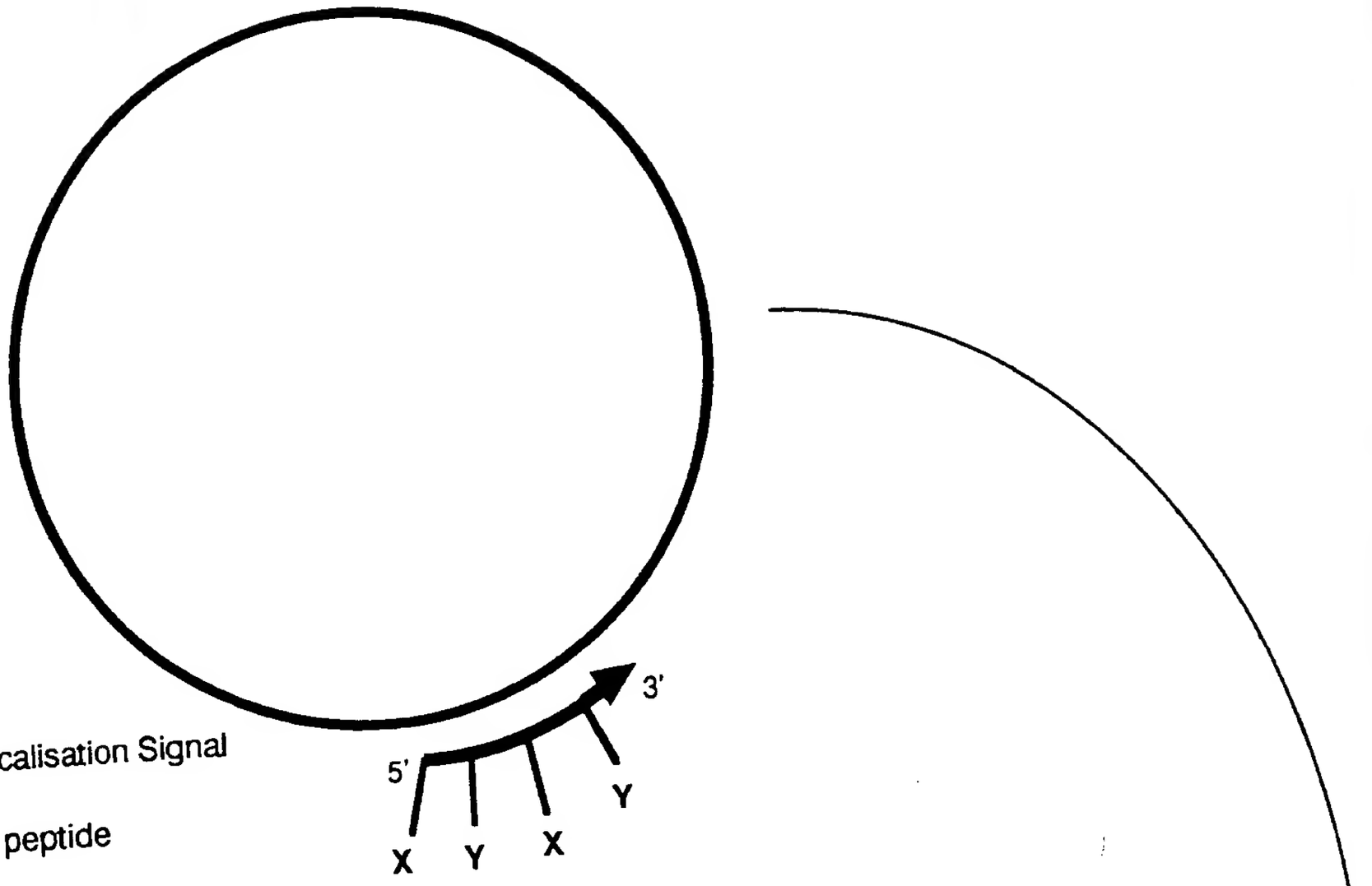


Figure I

Attachment of Ligands Through Primer Region

(a)



X = Nuclear Localisation Signal

Y = fusogenic peptide

(b)

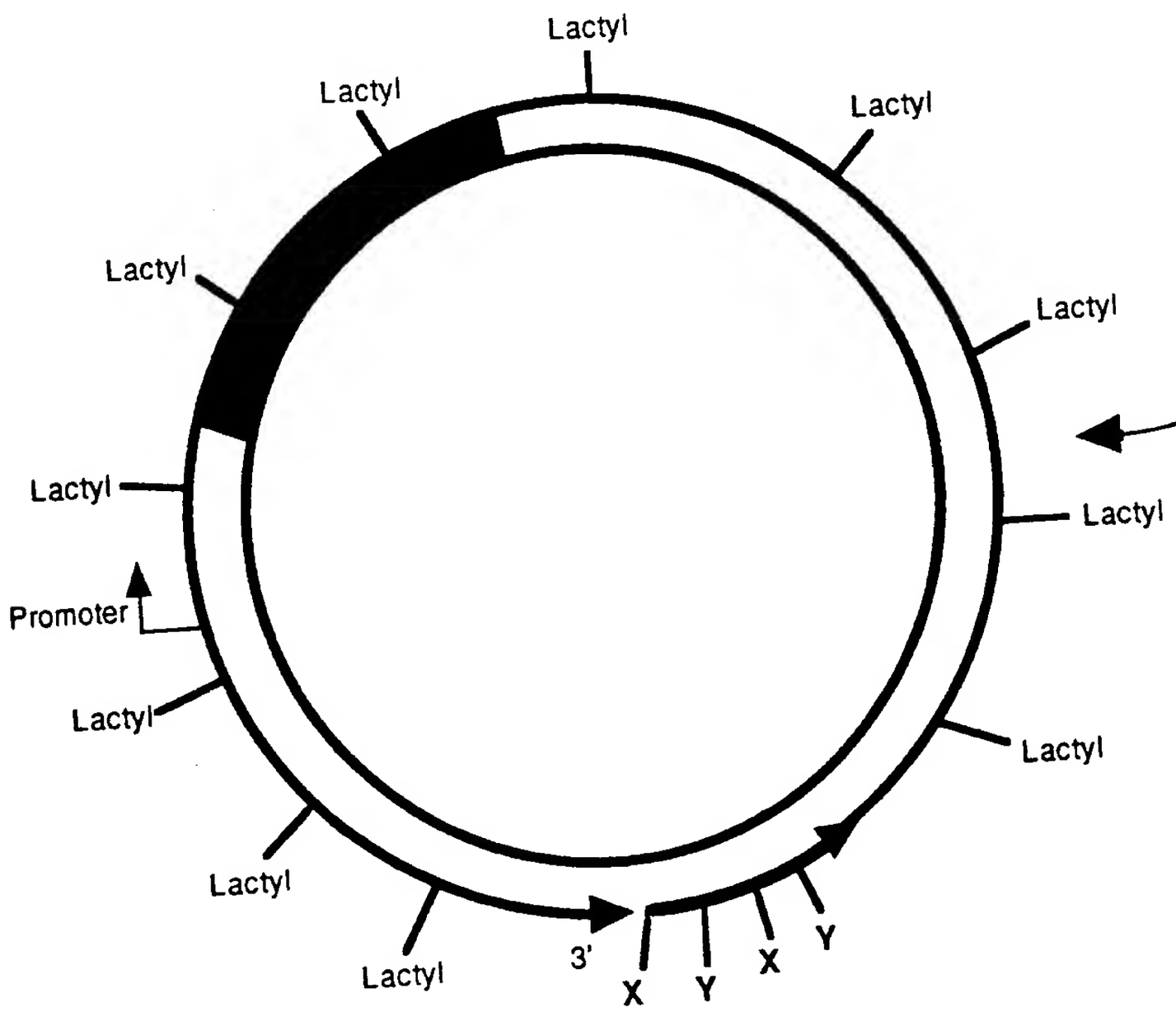
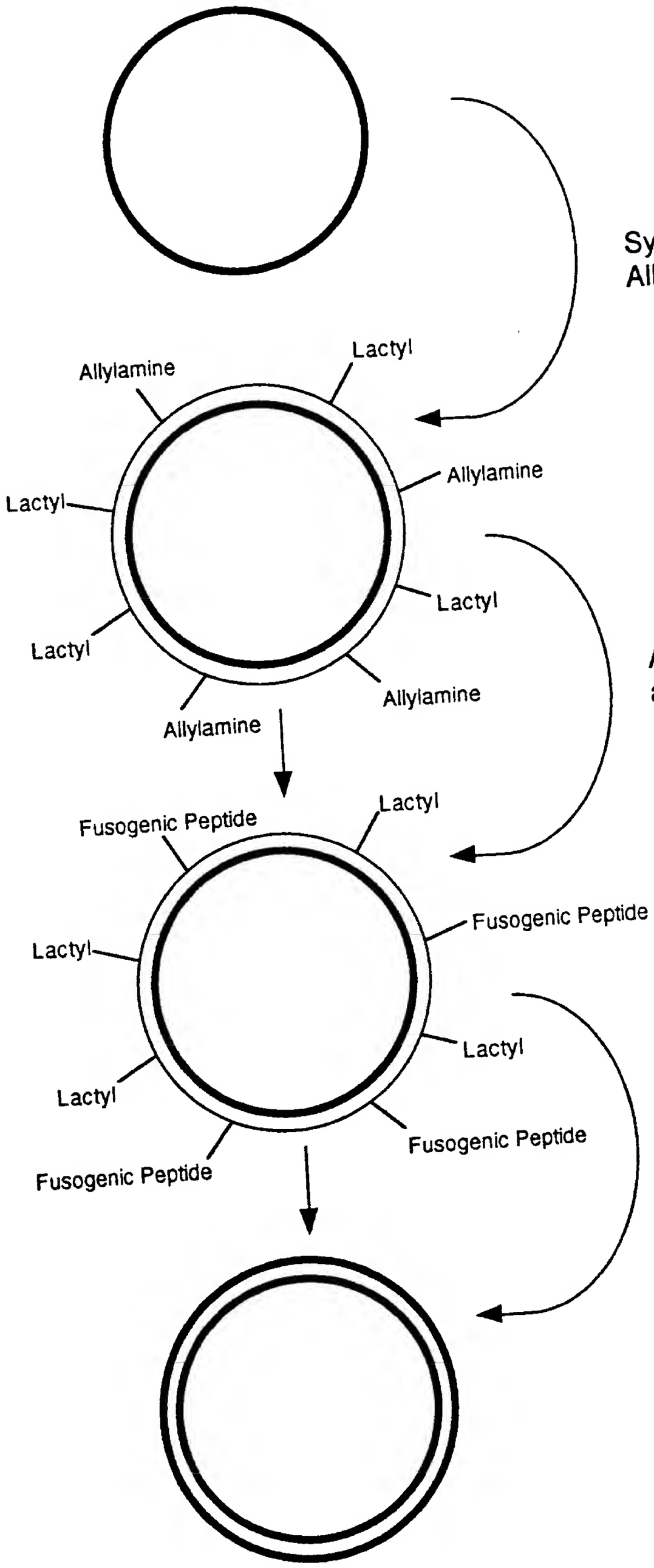


Figure 2
Attachment of Ligands by Incorporation of Modified Nucleotide Precursors



= DNA

= RNA

Synthesis of RNA using lactyl-UTP and Allylamine-UTP precursors

Attachment of fusogenice peptide through allylamine linkage

1) attachment of construct to cell surface
2) endocytosis of construct
3) release of construct from endosome by means of fusogenic peptide
4) Elimination of RNA moieties by RNaseH
5) Synthesis of complimentary DNA strand

Figure 3

Incorporation of Ligands through Modified Ribonucleotides

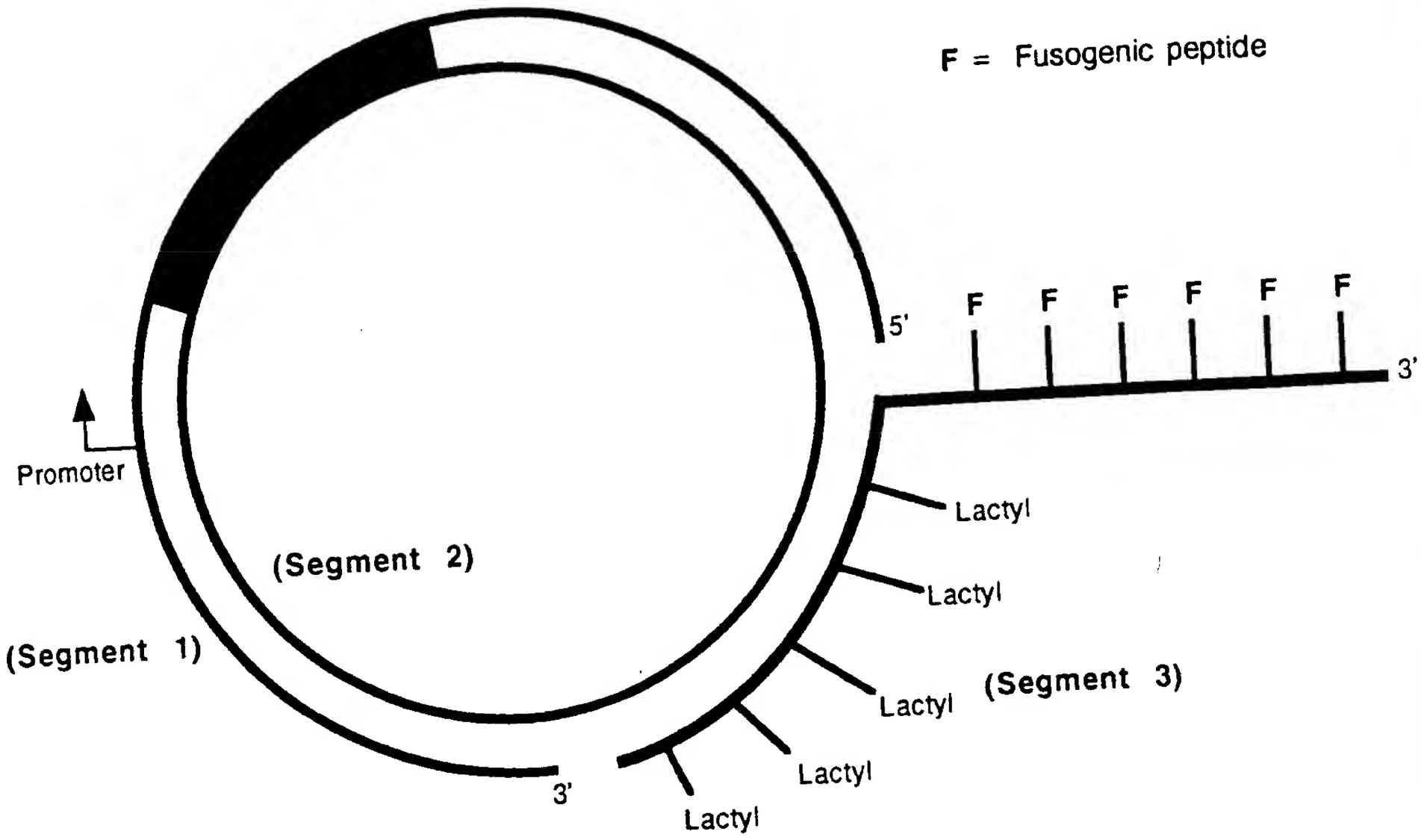


Figure 4

Attachment of Ligands through a 3' tail

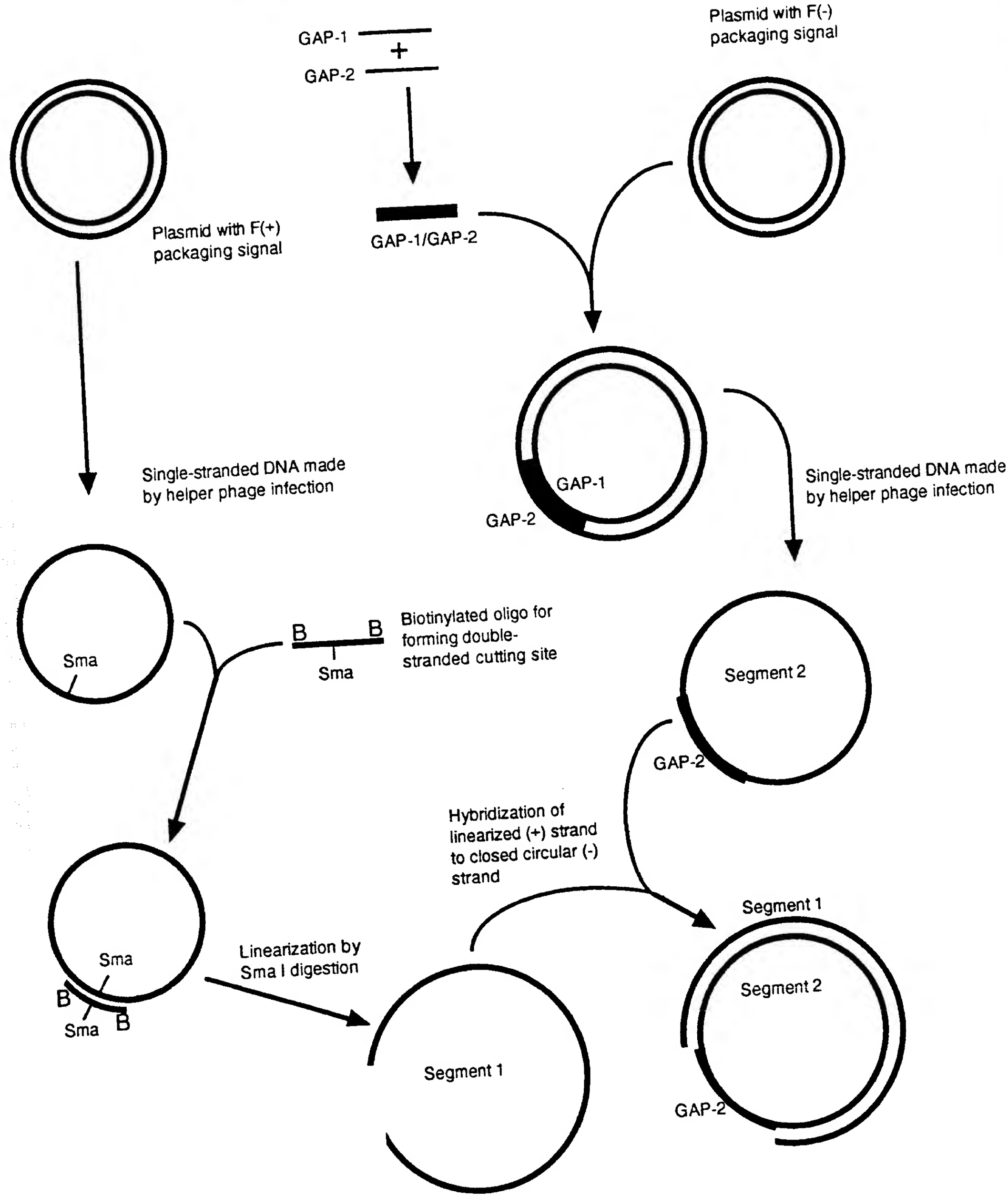


Figure 5

Preparation of Gapped Circle

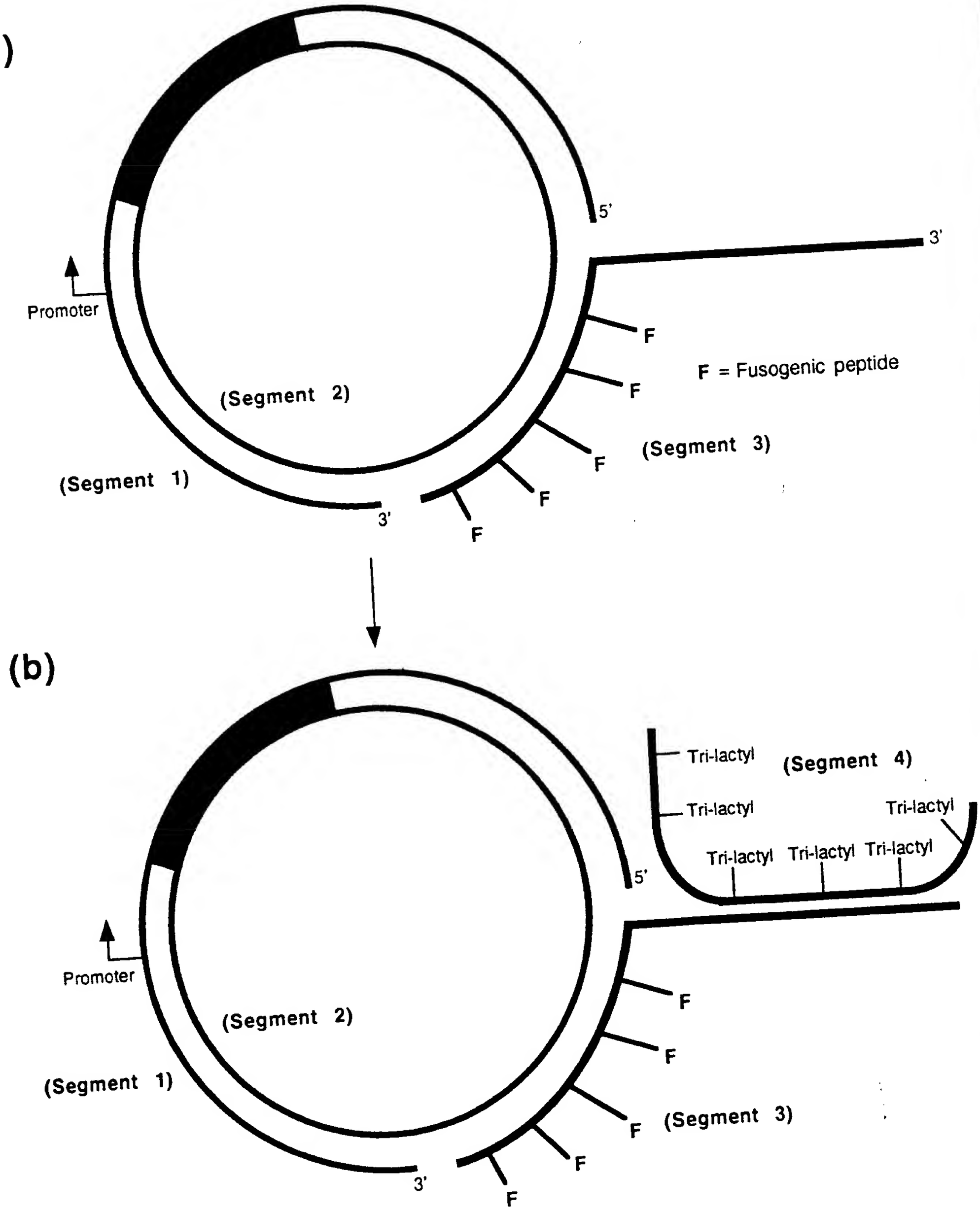
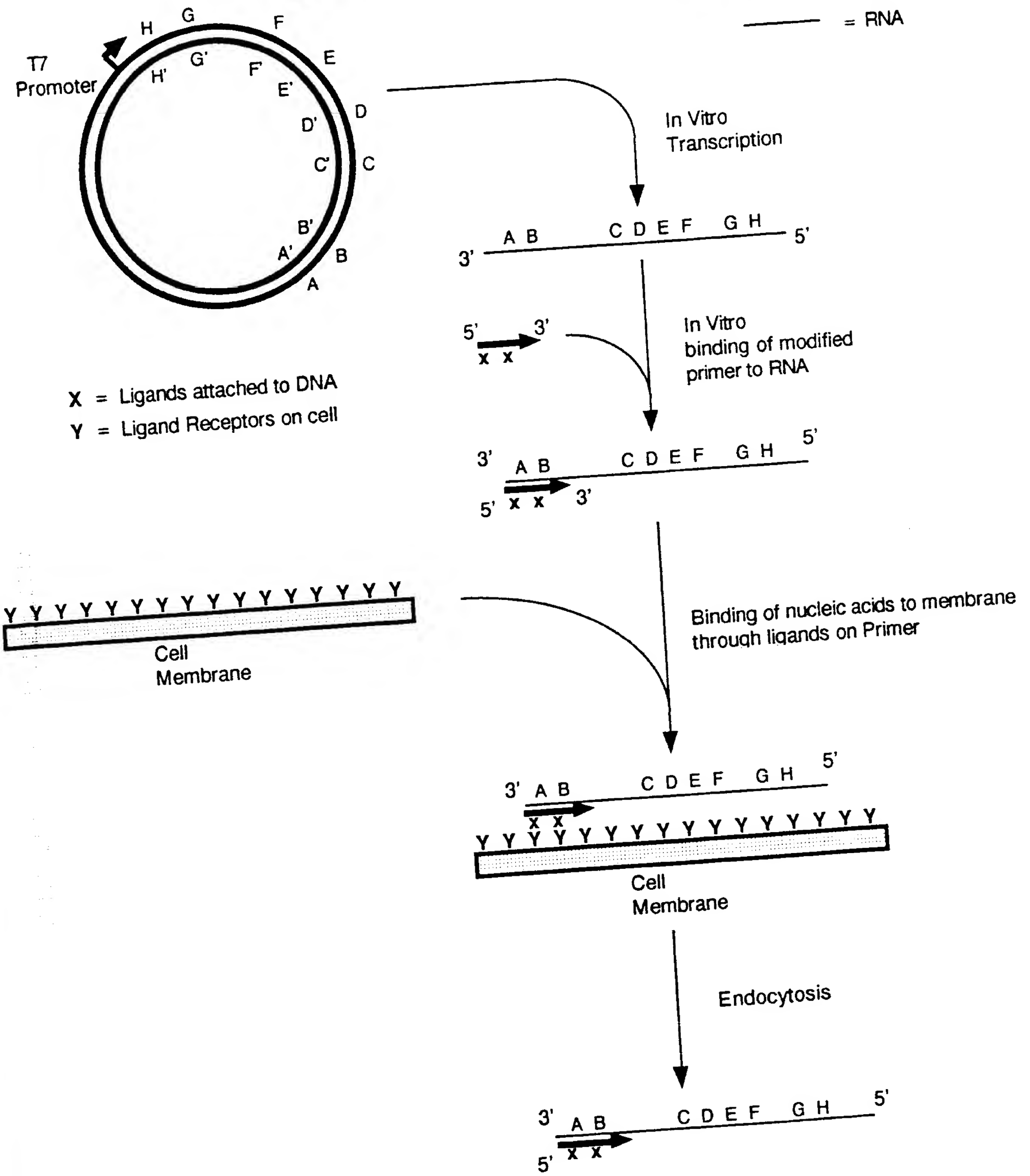


Figure 6

Attachment of Ligands through hybridization to a 3' tail



(Continued in Figure 8)

Figure 7

RNA with Ligands on Primer

Continued from Figure 7

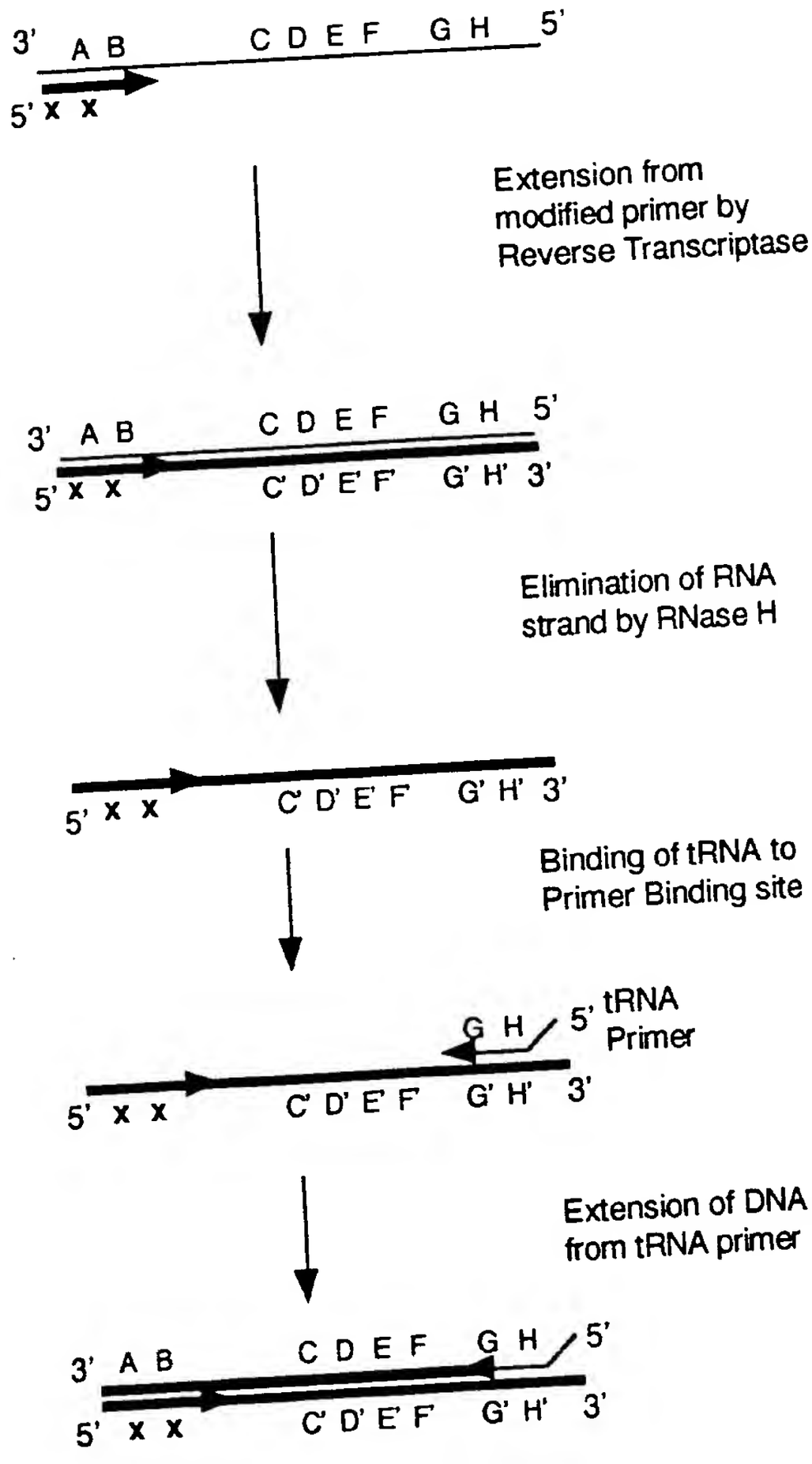
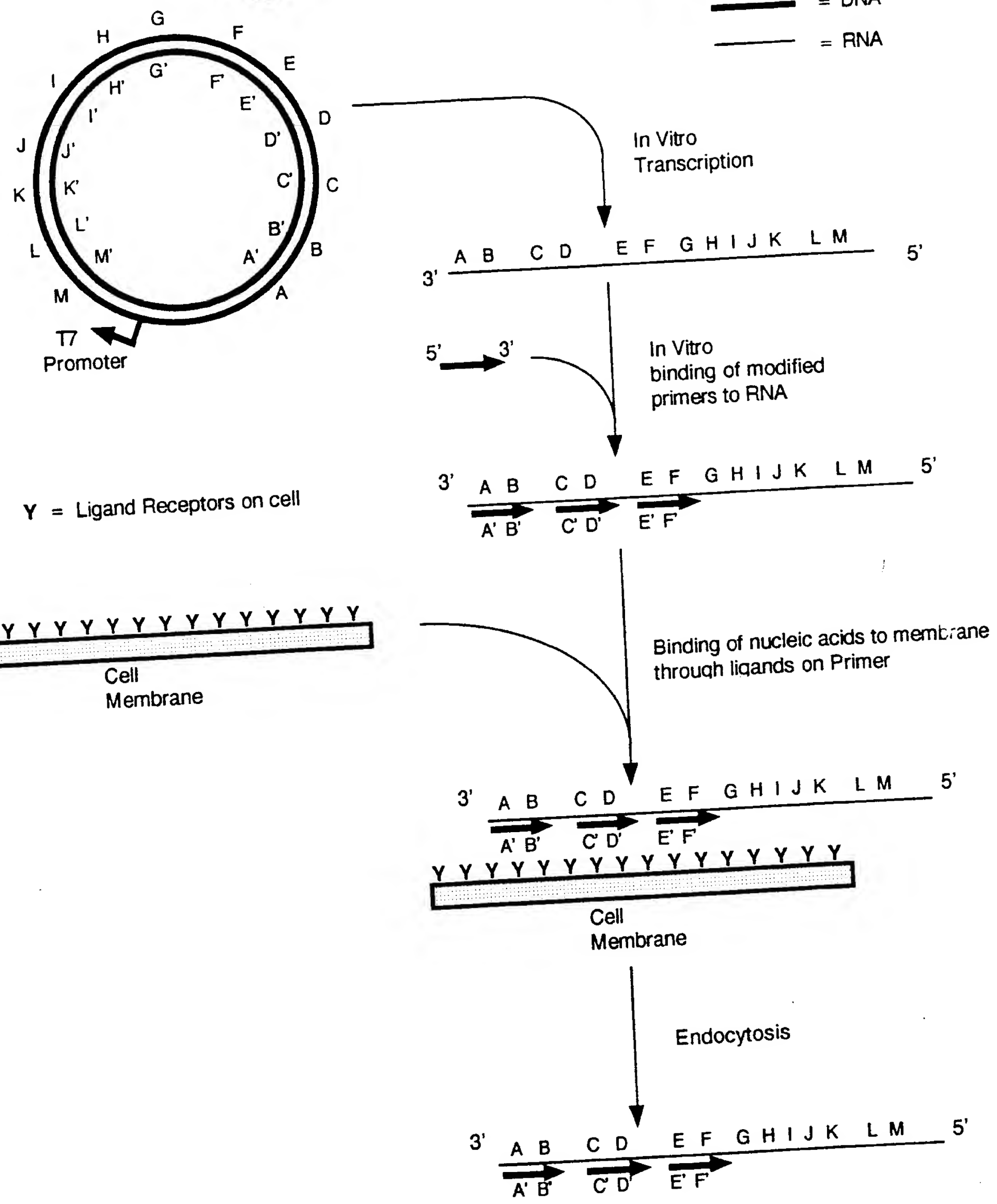


Figure 8

RNA with Ligands on Primer (Continued)



(Continued in Figure 10)

Figure 9

RNA with Ligands on Multiple Primers

Continued from Figure 9

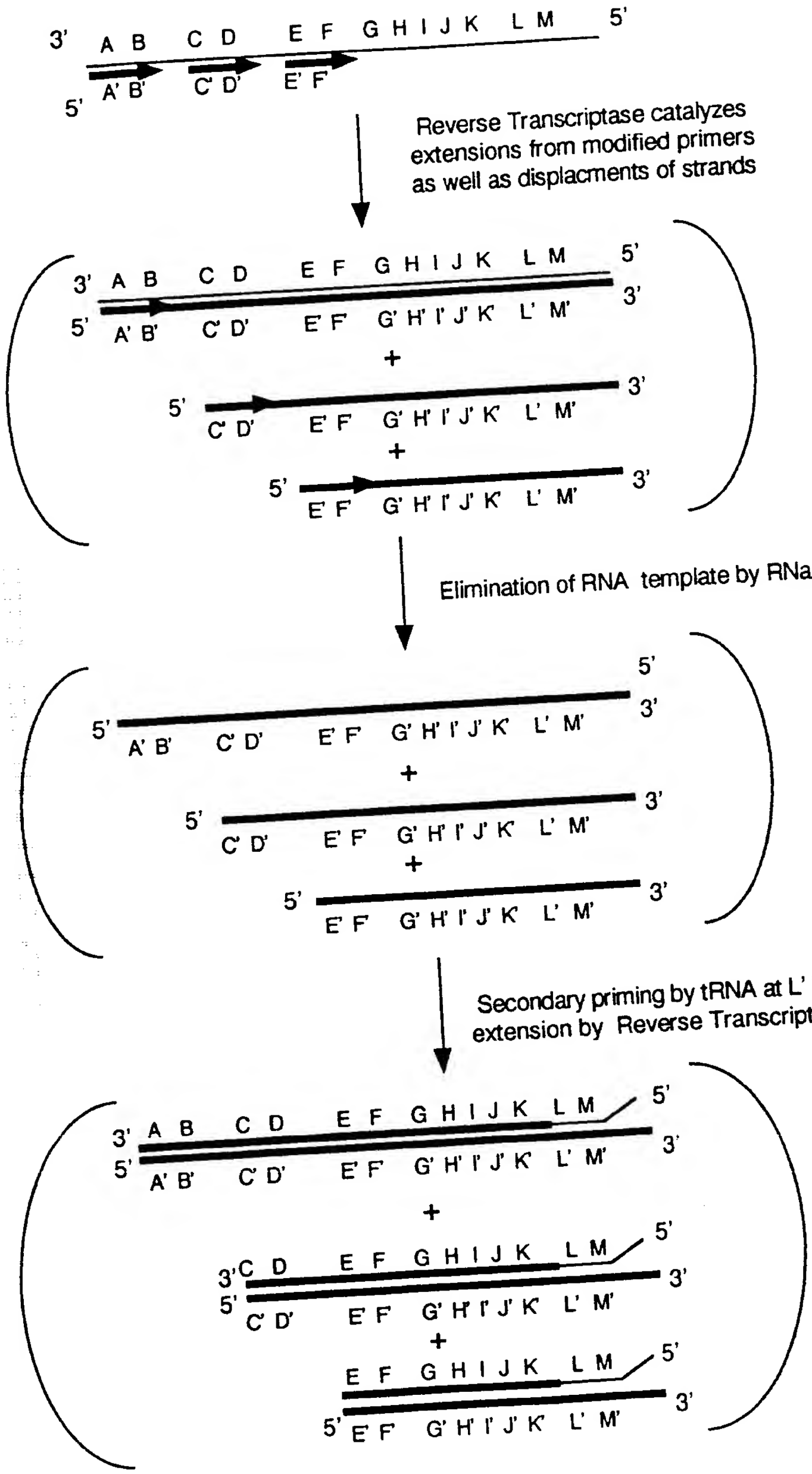
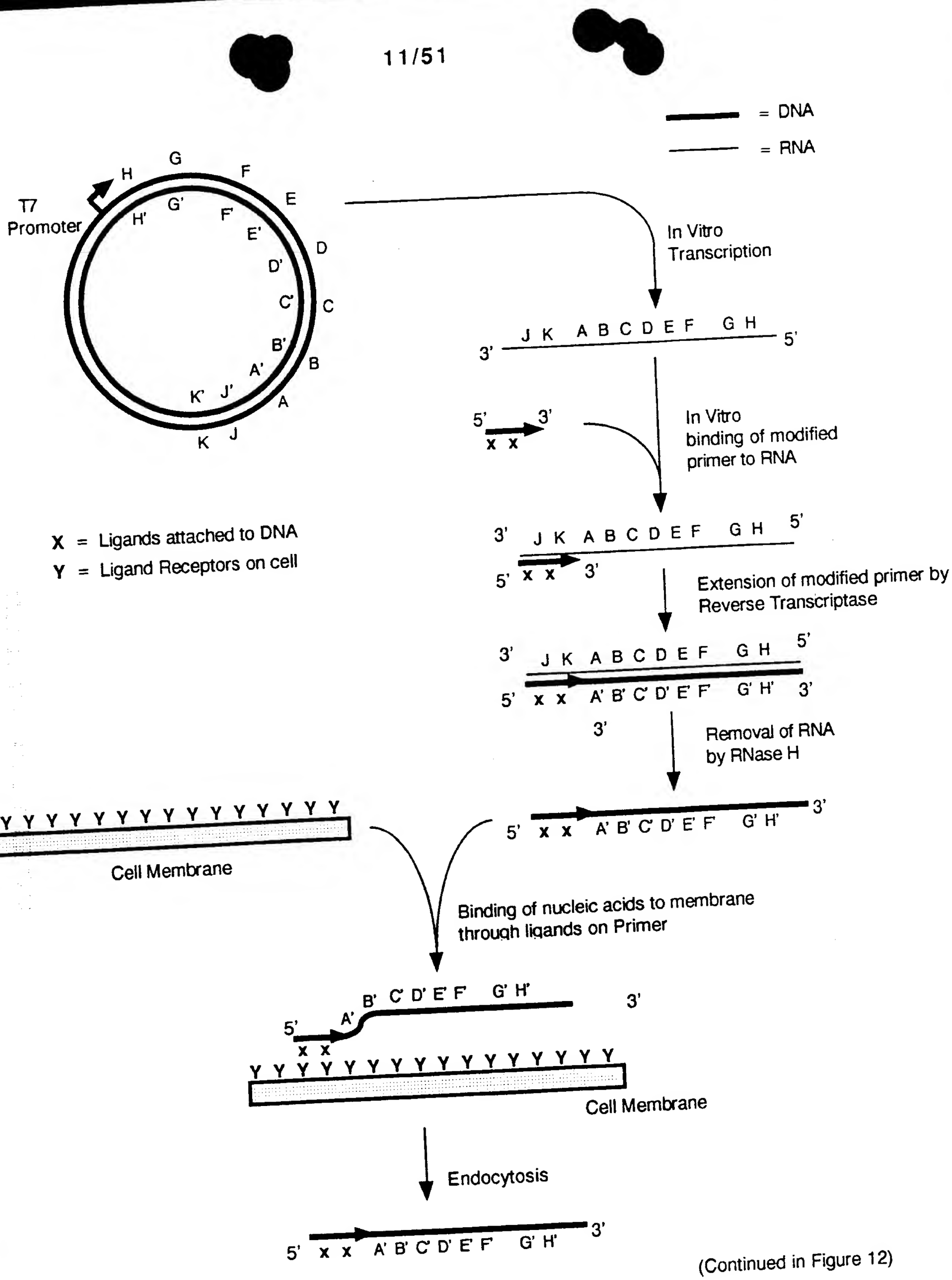


Figure 10

RNA with Ligands on Multiple Primers (Continued)



(Continued in Figure 12)

Figure 11

Single-stranded DNA with attached Ligands

Continued from Figure 11

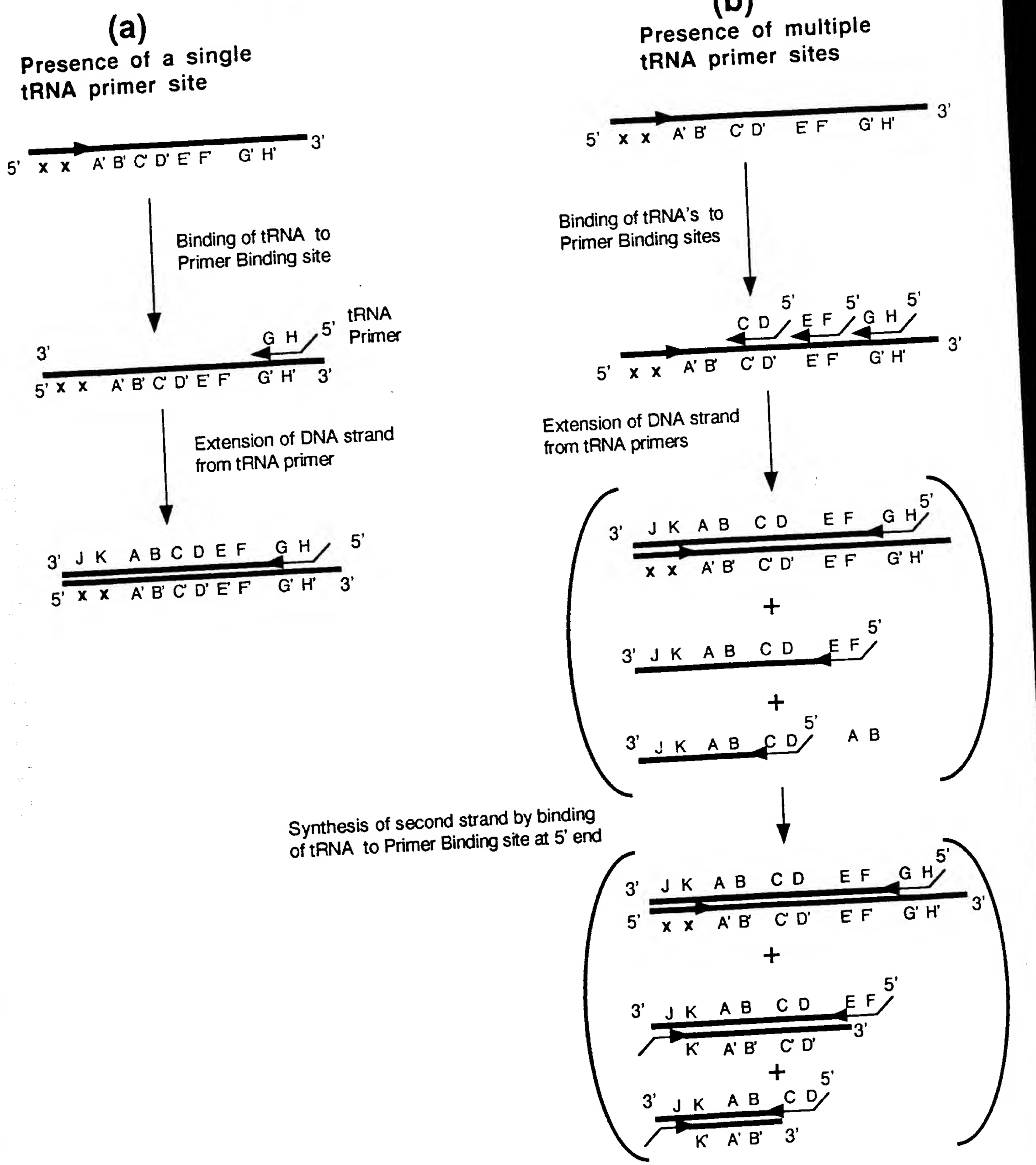
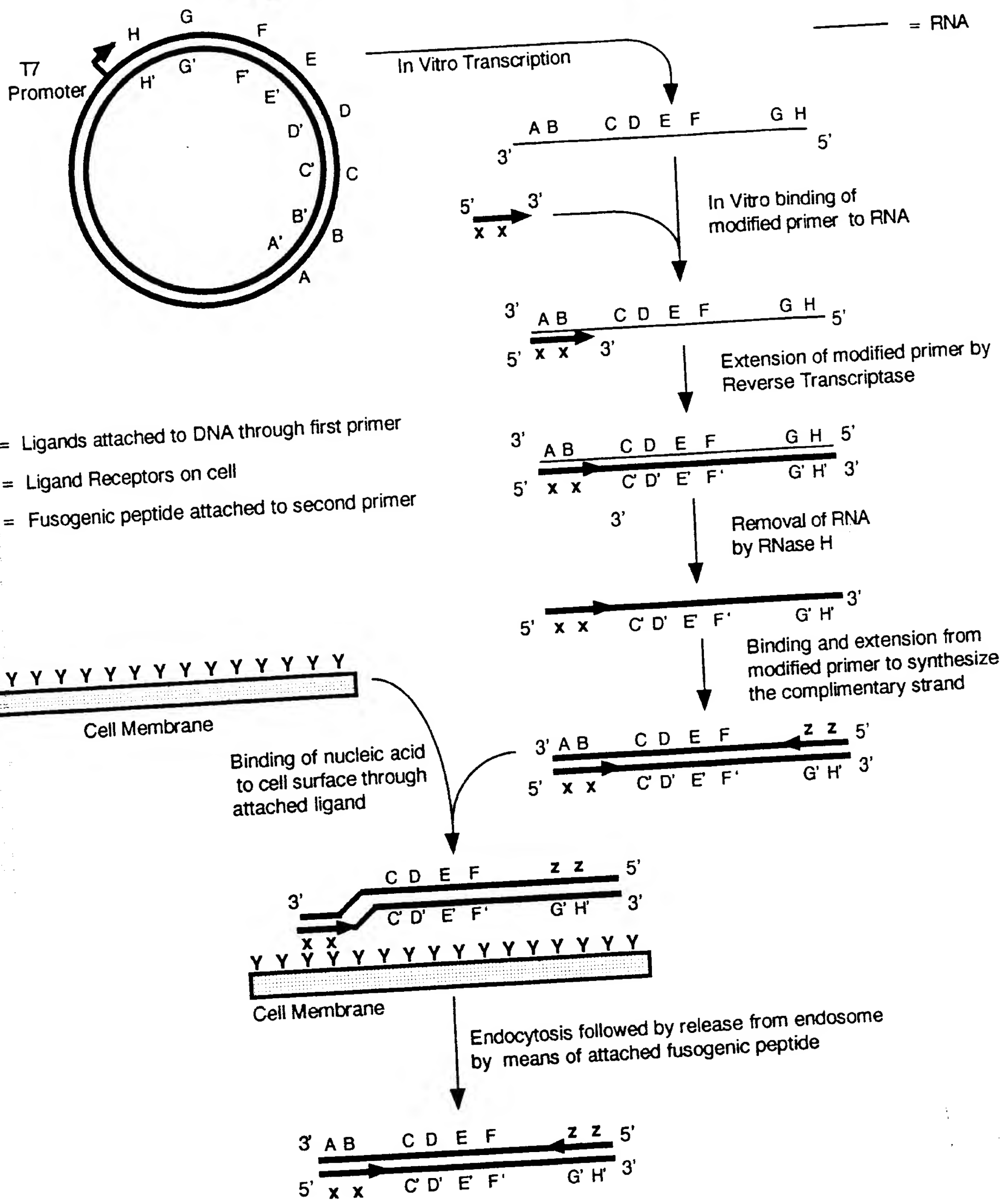


Figure 12

Single-stranded DNA with attached Ligands (continued)



X = Ligands attached to DNA through first primer

Y = Ligand Receptors on cell

Z = Fusogenic peptide attached to second primer

Figure 13

Linear Double-stranded DNA with attached Moieties on each strand

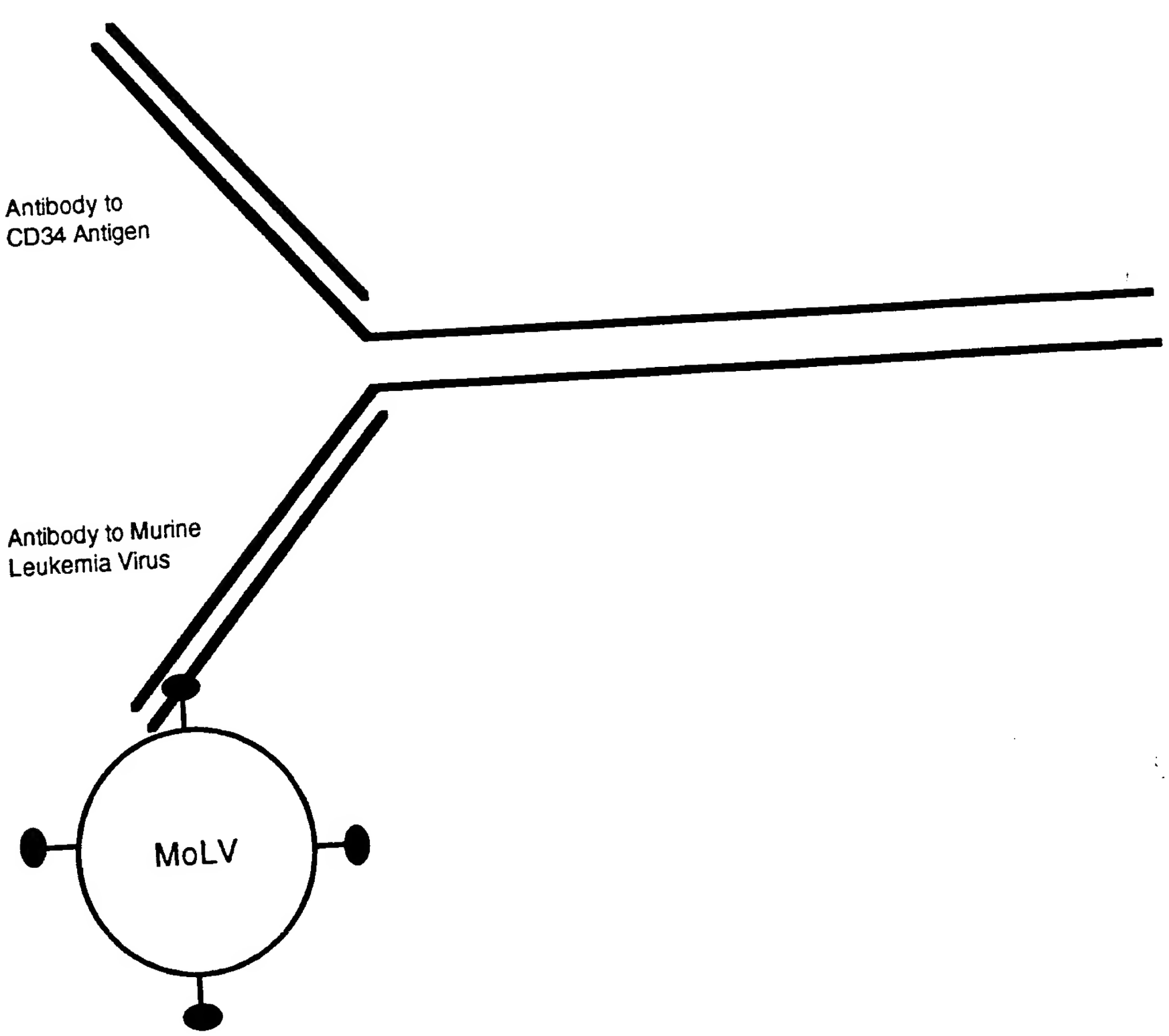


Figure 14

Enhanced Delivery of Retroviral Vector
to Haematopoeitic Stem Cell

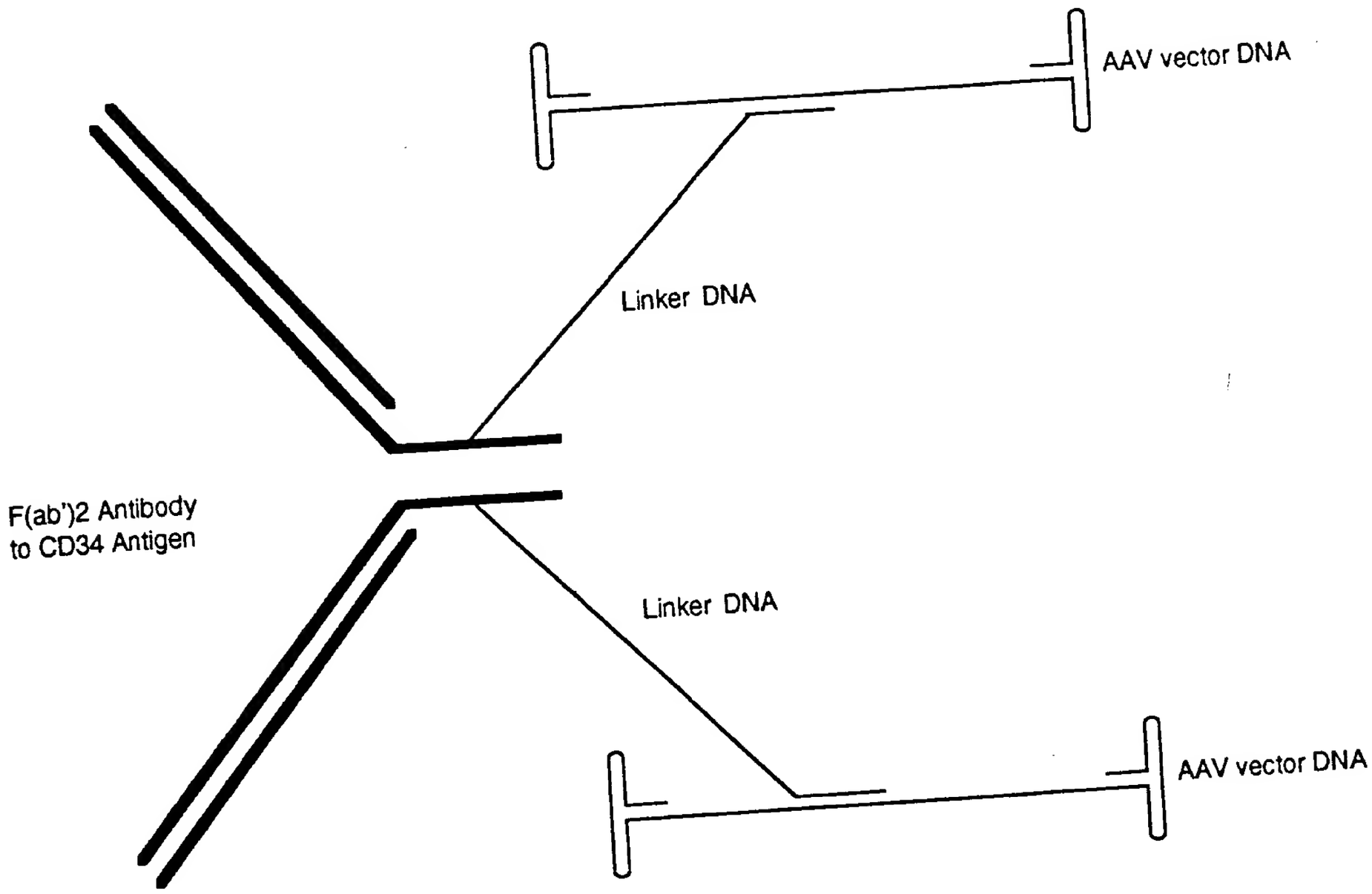
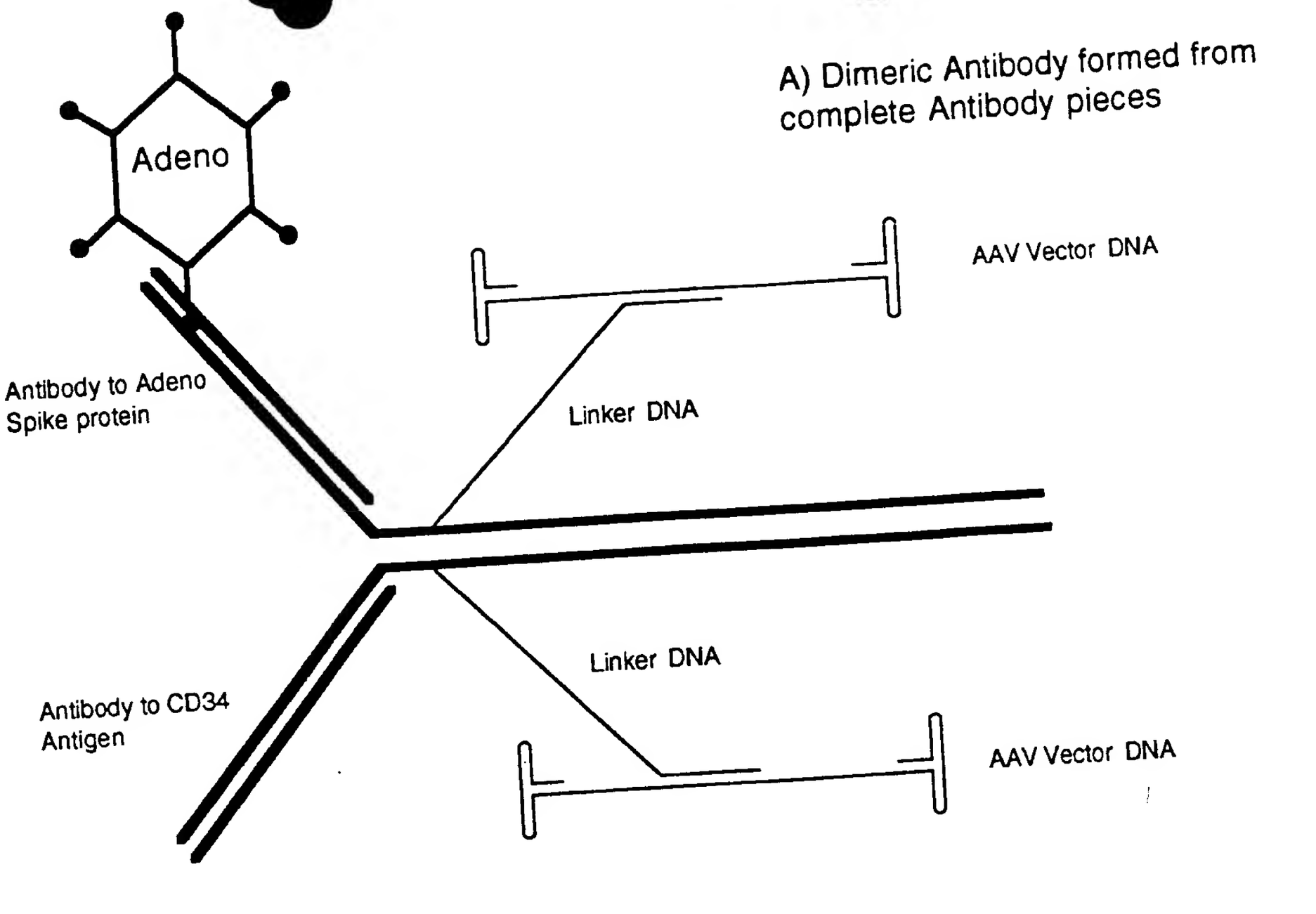


Figure 15

Enhanced Delivery of Vector
DNA to Haematopoeitic Stem Cell

A) Dimeric Antibody formed from complete Antibody pieces



B) Dimeric Antibody formed from F(ab') fragments

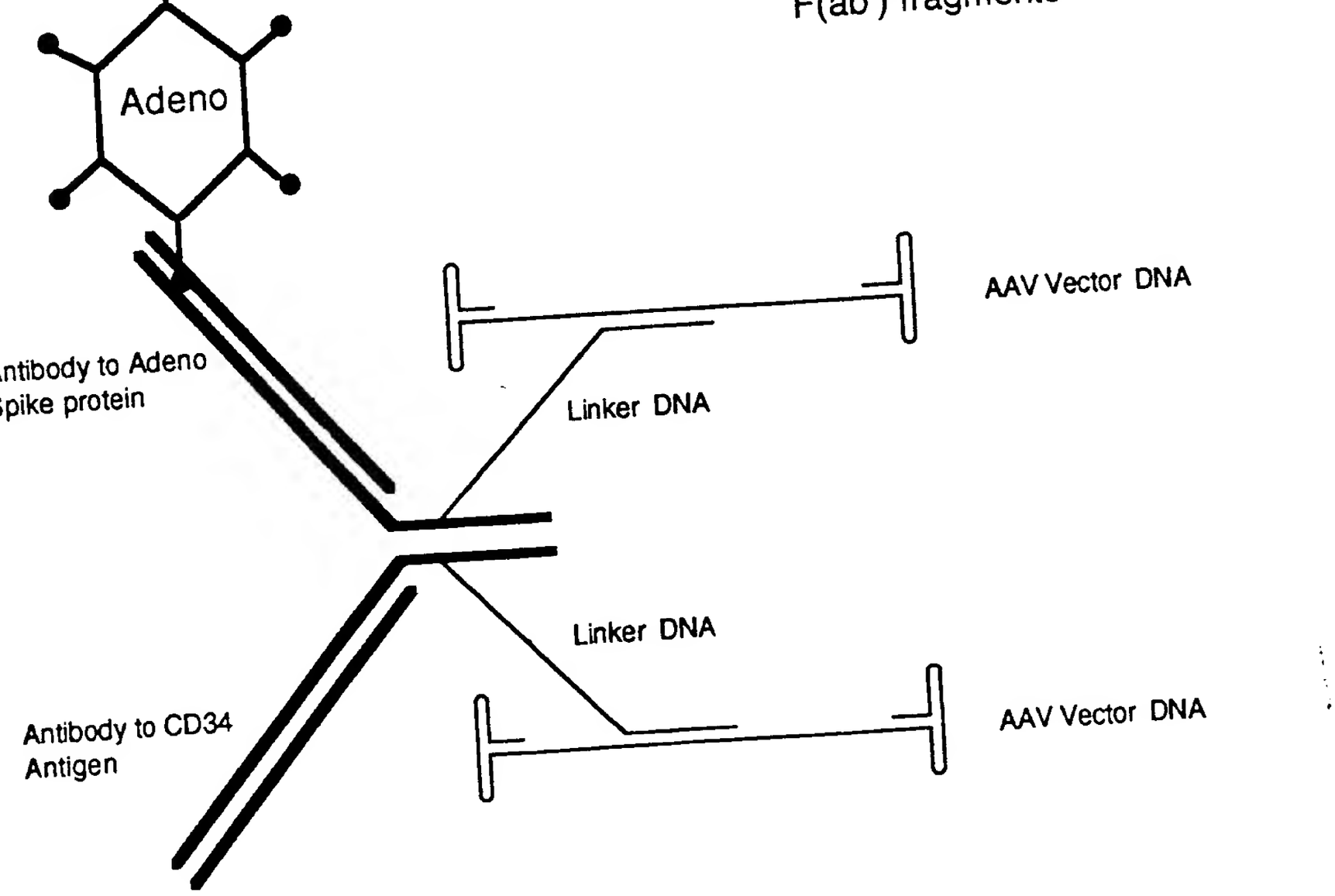


Figure 16

Covalent Attachment of vector DNA to Dimeric Antibody

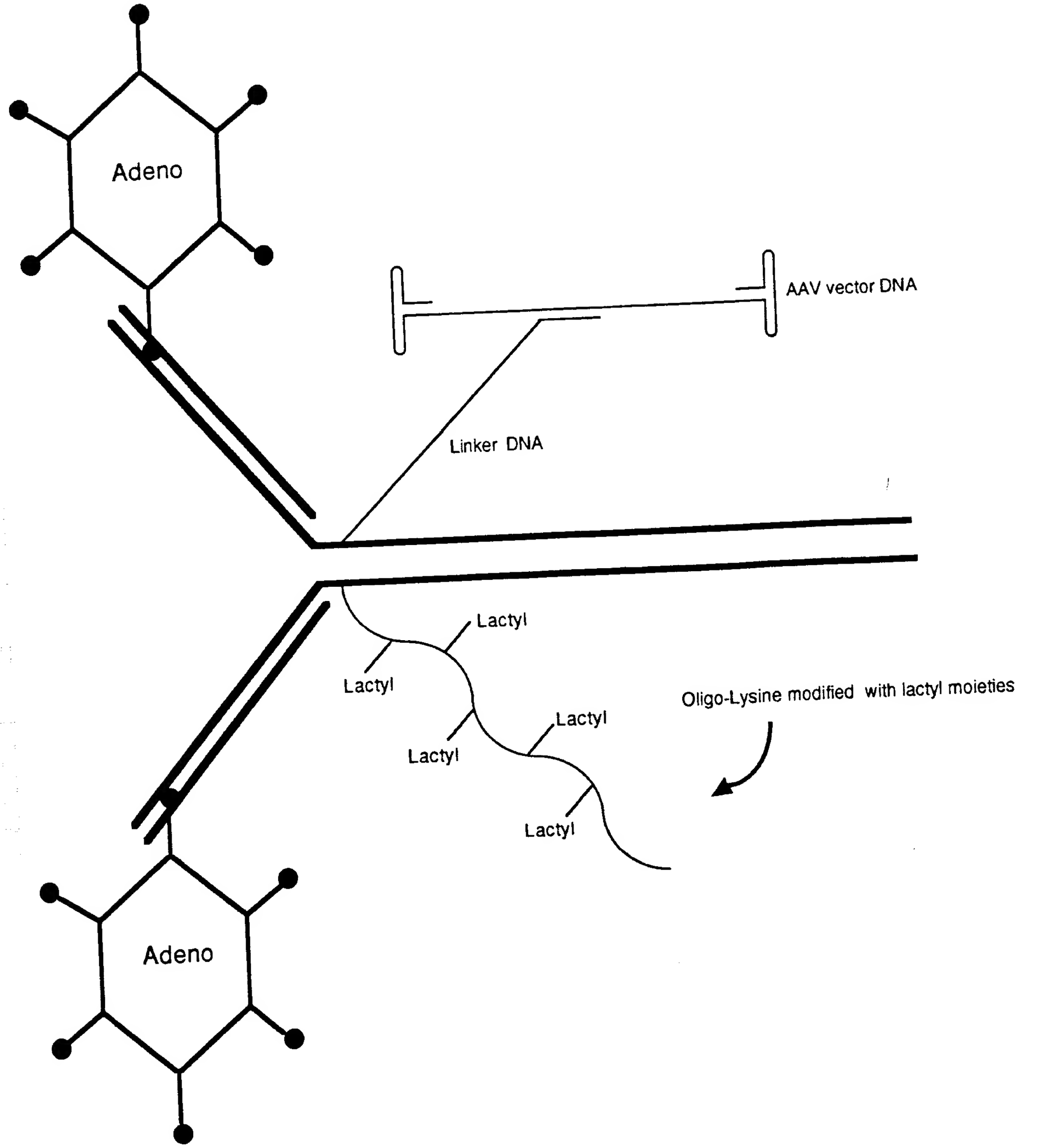


Figure 17

Covalent attachment of Modified DNA to a Monovalent Antibody

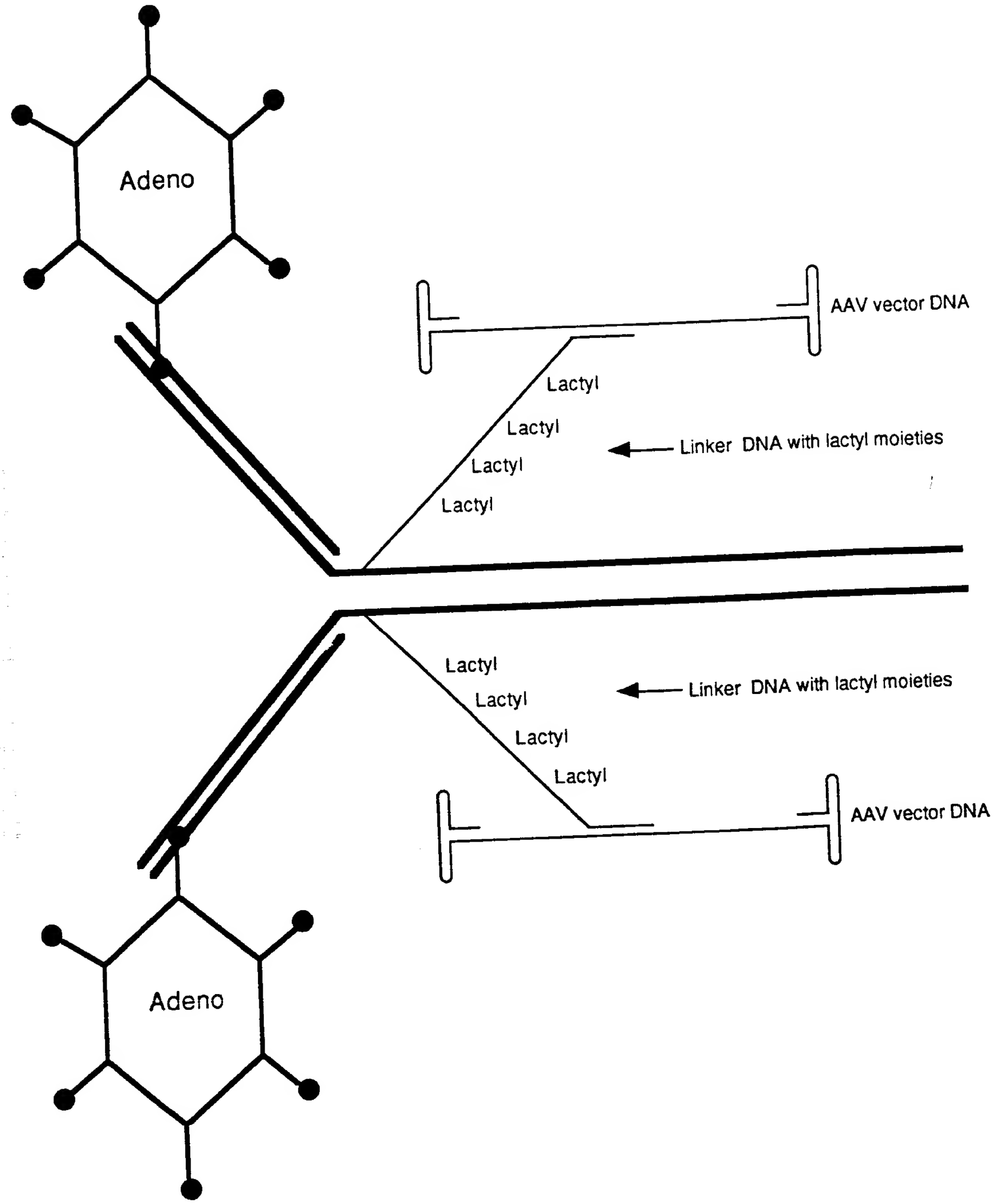
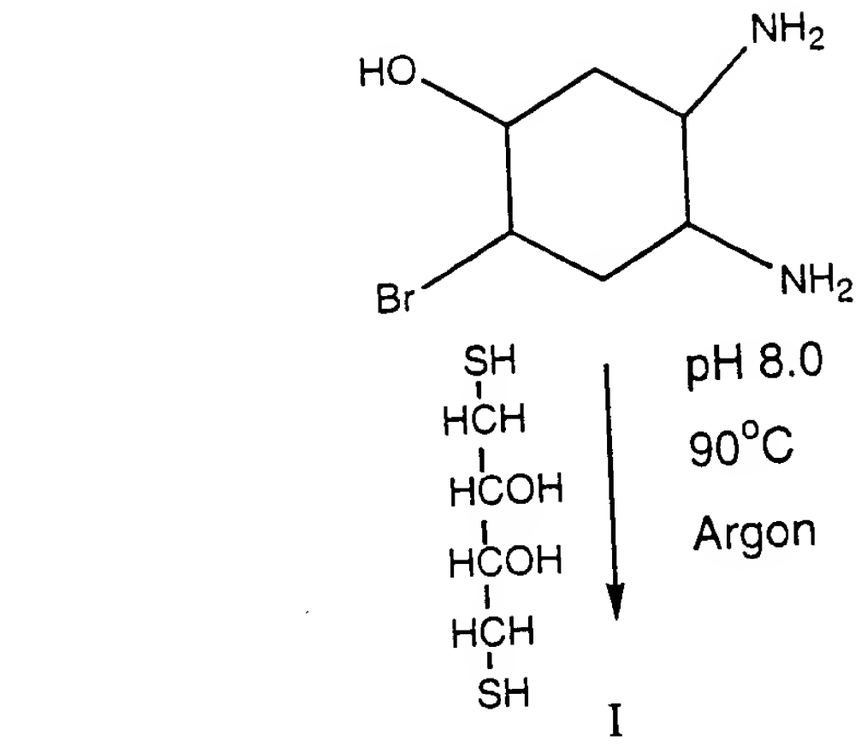
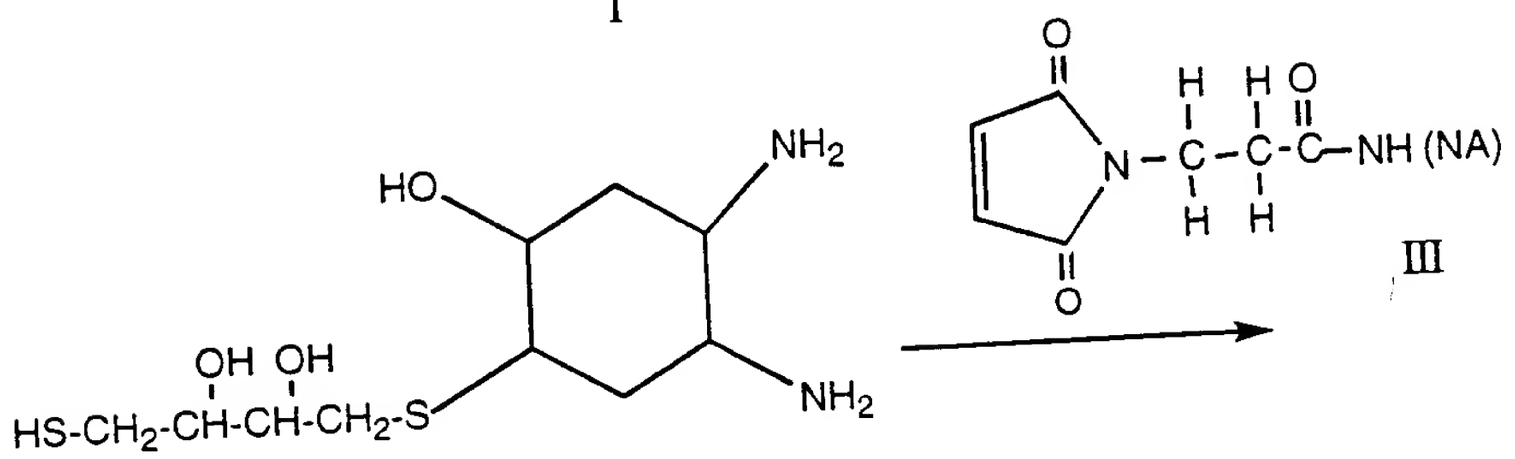


Figure 18

Modified DNA used as a Binder

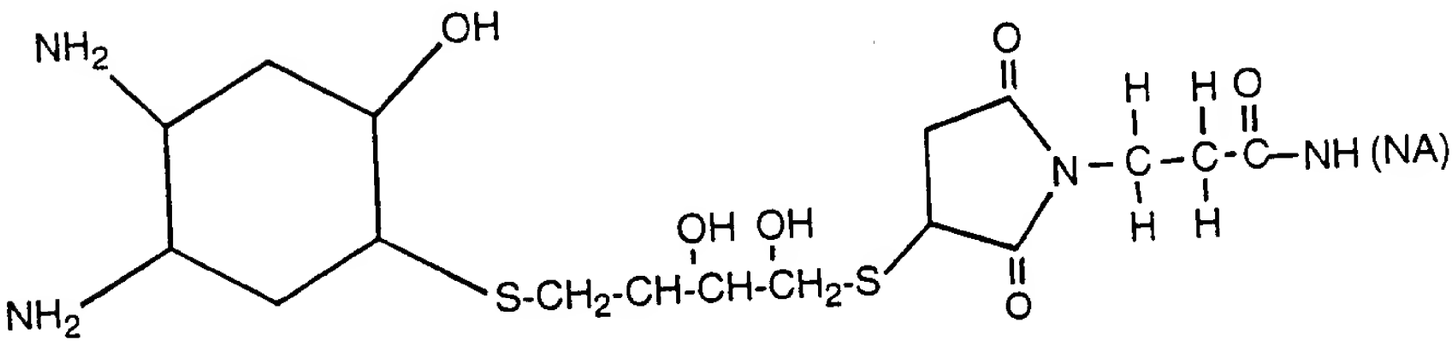


I

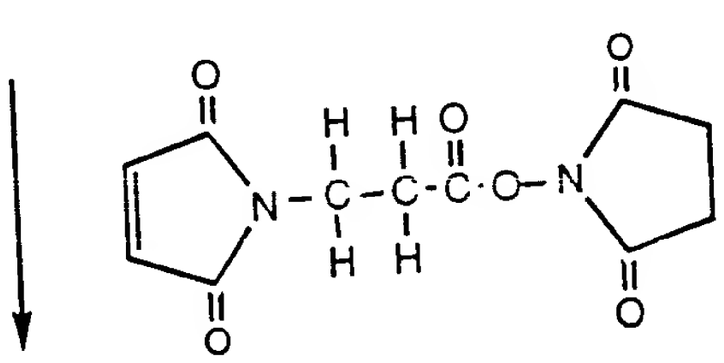


II

III



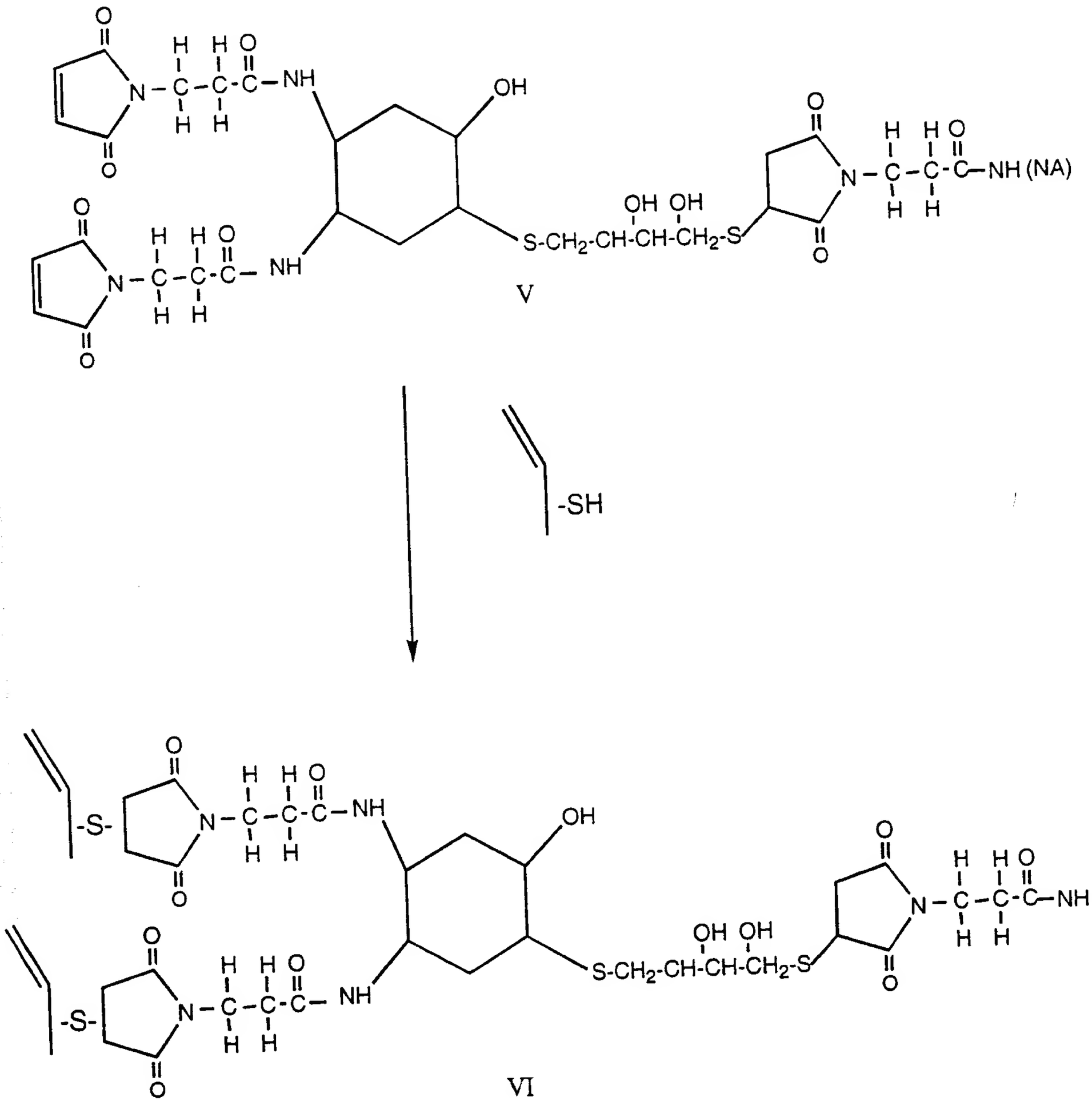
IV



(continued in Figure 20)

Figure 19
Synthetic Steps for Creation of Antibodies With Nucleic Acid Moieties Attached

(Continued from Figure19)



V

VI

Figure 20
Continuation of Synthetic Steps

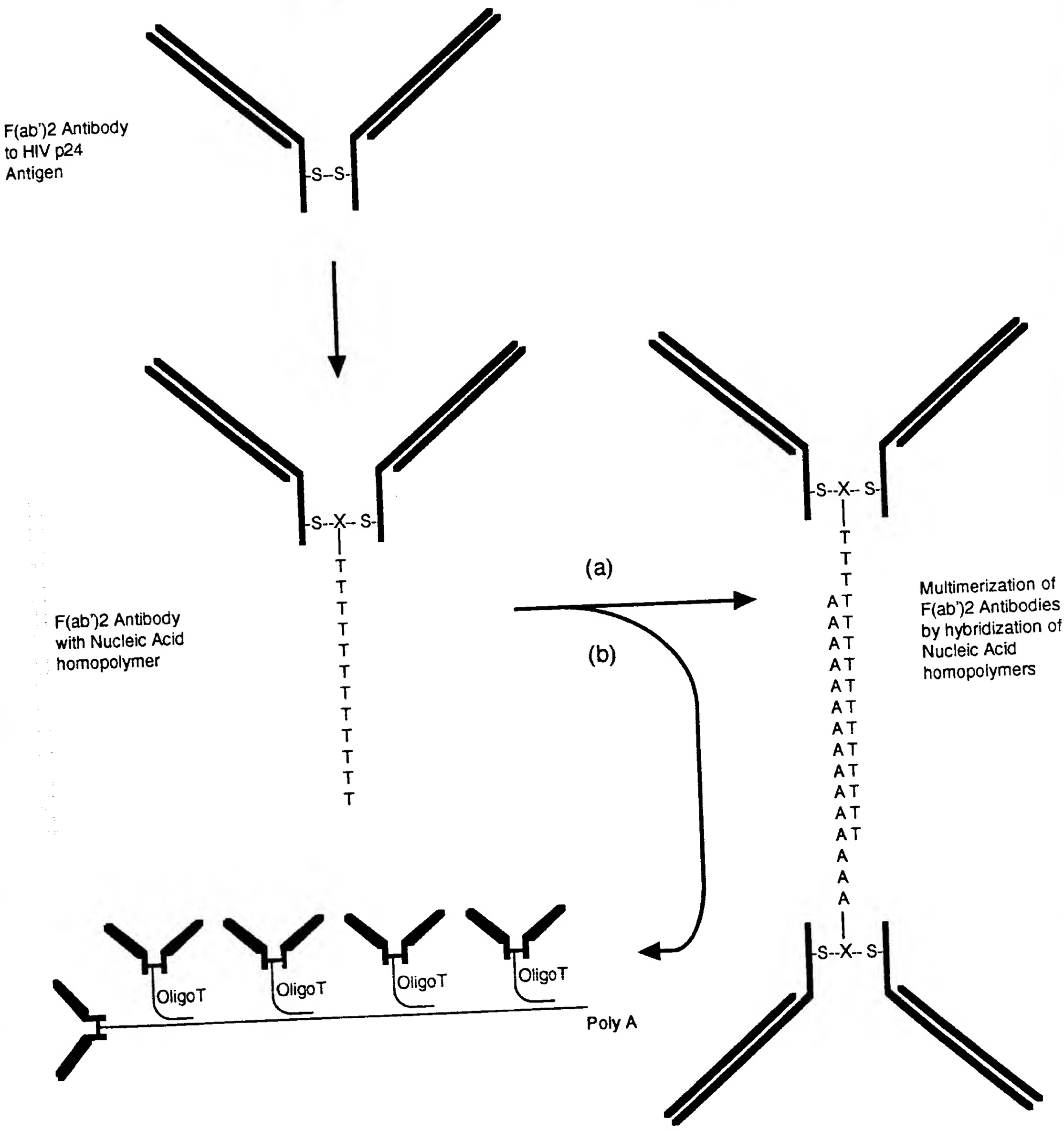


Figure 21

Enhanced Binding of Antibodies to Antigens by Multimerization

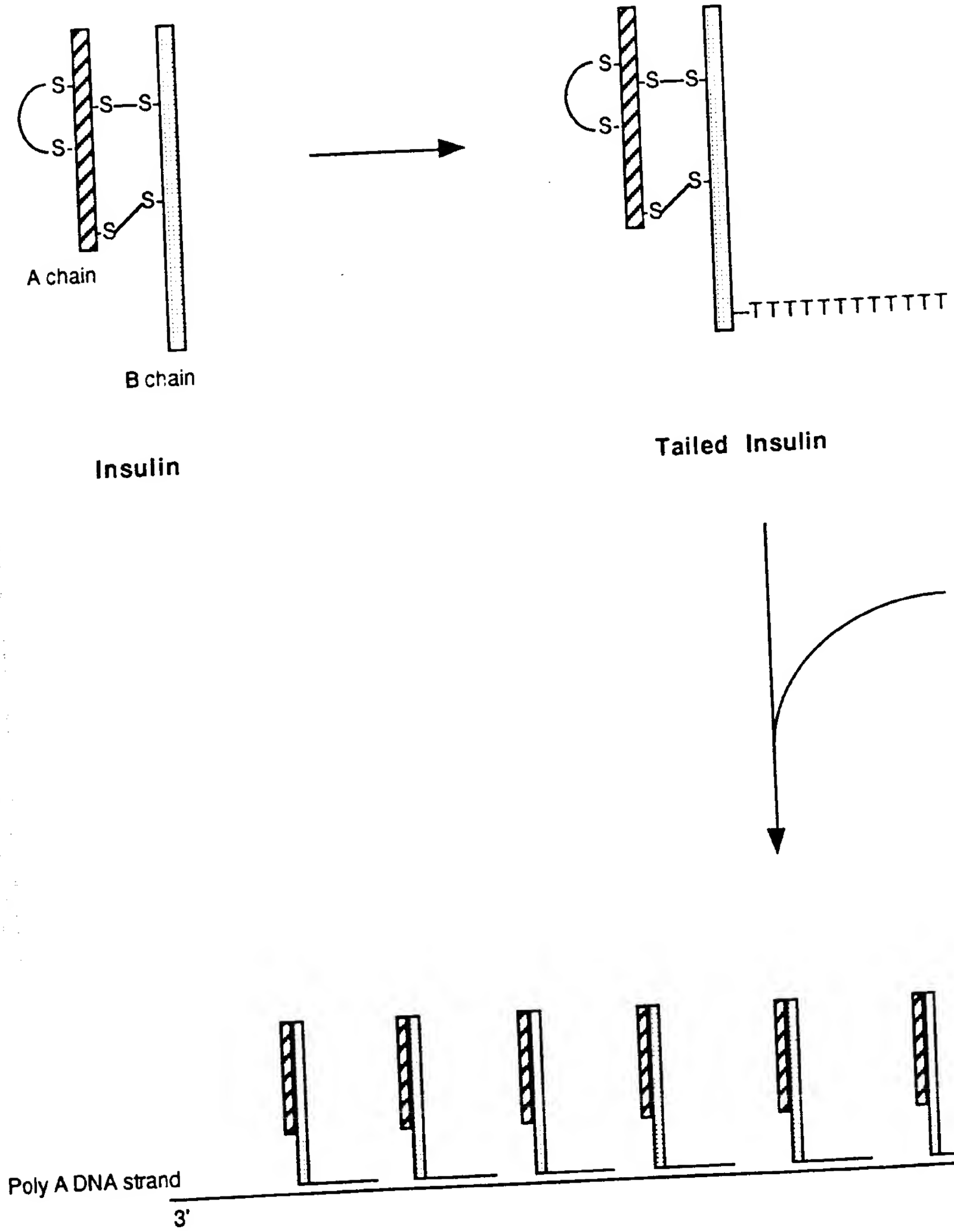


Figure 22

High Affinity Multi-Insulin Soluble Complex

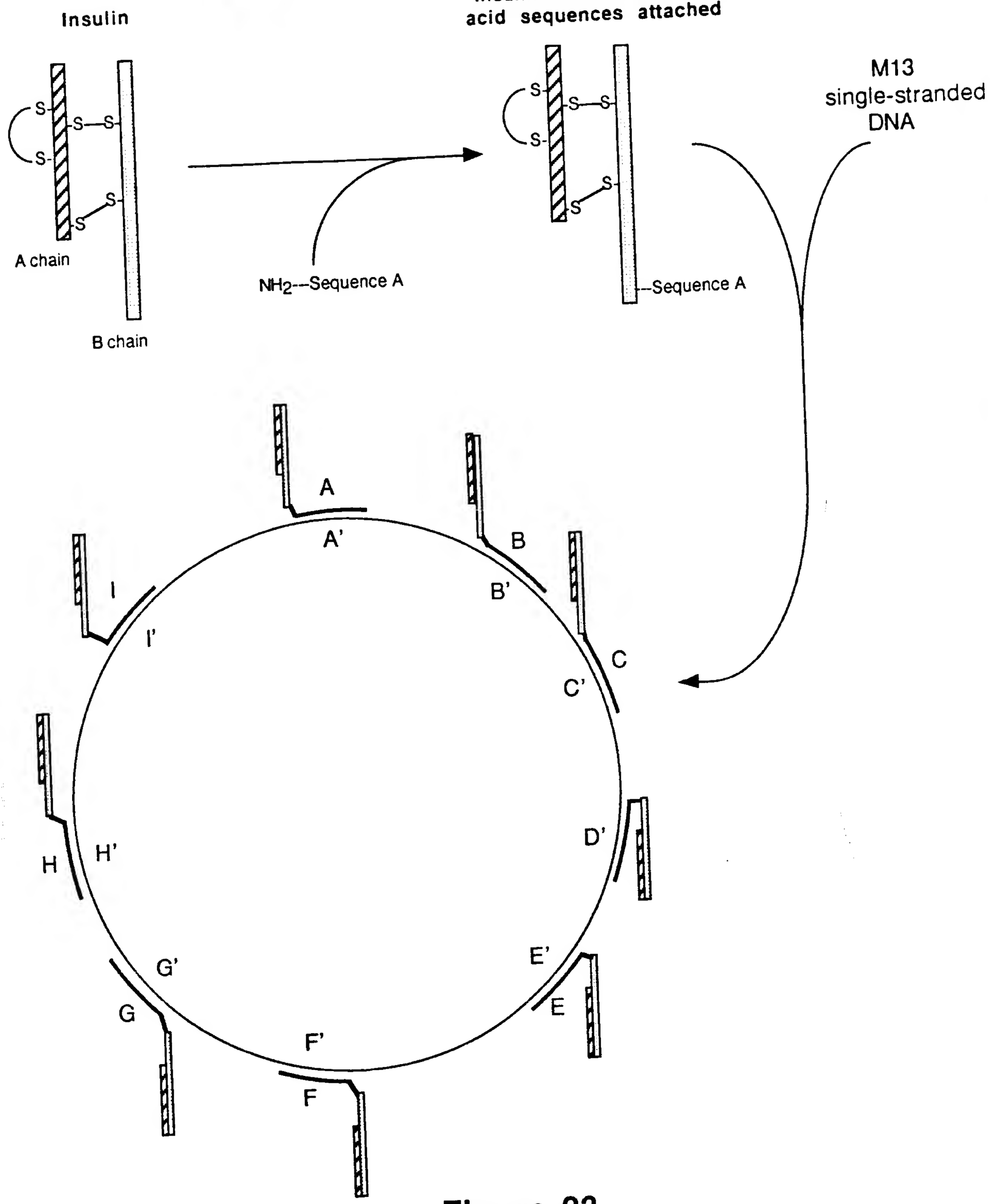


Figure 23

Multimerization of Insulin molecules by hybridization to discrete Sequences

Intron insertion site

(A) ----TGCTCTCTAAGGGTCTACTC----
----ACGAGAGATTCCCAGATGAG----

T7 RNA Polymerase Sequence

Splice Donor Site

Splice Acceptor site

(B) ----CTCTAAGGTAAATAT - - - - - - - - - TGTATTTTAGATTCAA----
----GAGATTCCATTTATA - - - - - - - - - ACATAAAATCTAAGTT----

SV40 Intron Sequence

(C) ----**TGCTCTCTAAG**GTAAATAT - - - - - - - - TGTATTTTAG**GGTCTACTC**----
----**ACGAGAGATTC**CATTTATA - - - - - - - - ACATAAAATC**CCAGATGAG**----

Insertion of SV40 Intron into polymerase coding sequence

Splice Donor Site

Splice Acceptor site

(D) ----**UGCUCUCUAAG**GUAAAUAU - - - - - - - - UGUAUUUUAG**GGUCUACUC**---

mRNA transcript containing intron

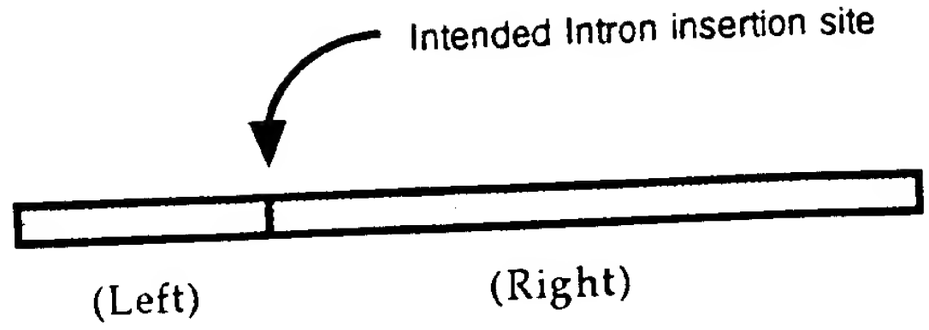
(E) ----UGCUCUCUAAGGGUCUACUC---

mRNA transcript after splicing has normal T7 Sequence

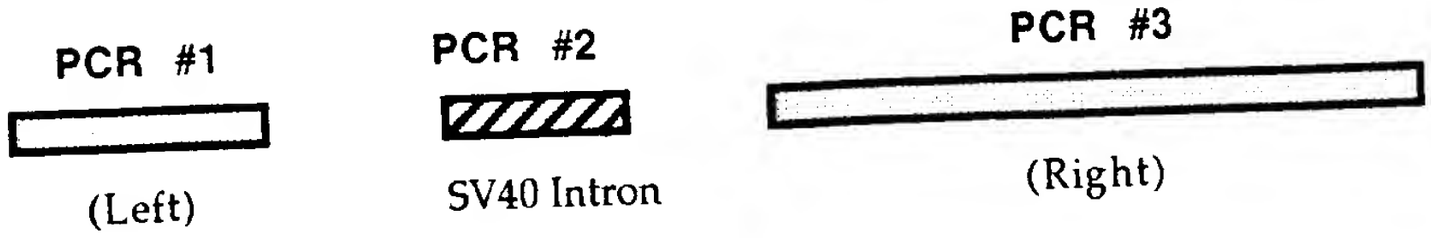
Figure 24

Fusion of Intron into T7 RNA Polymerase Coding Sequence

Normal T7 RNA polymerase coding sequence



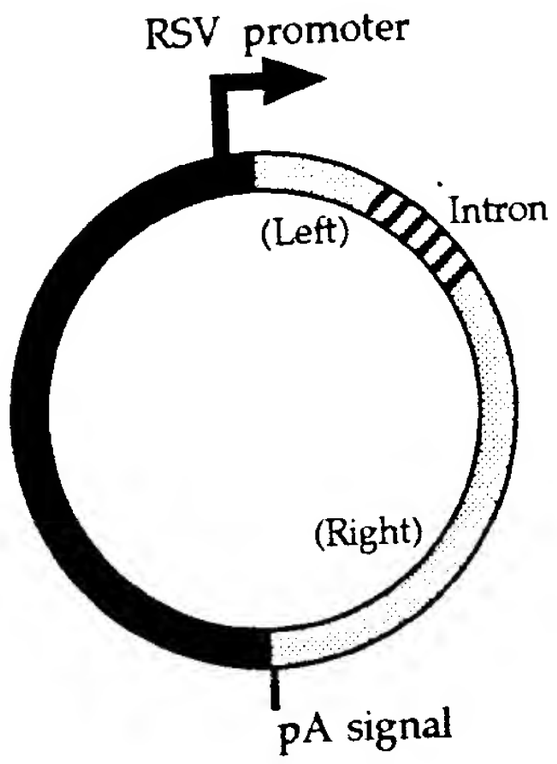
Synthesis of fragments by PCR Amplification of T7 or SV40 templates



(A)

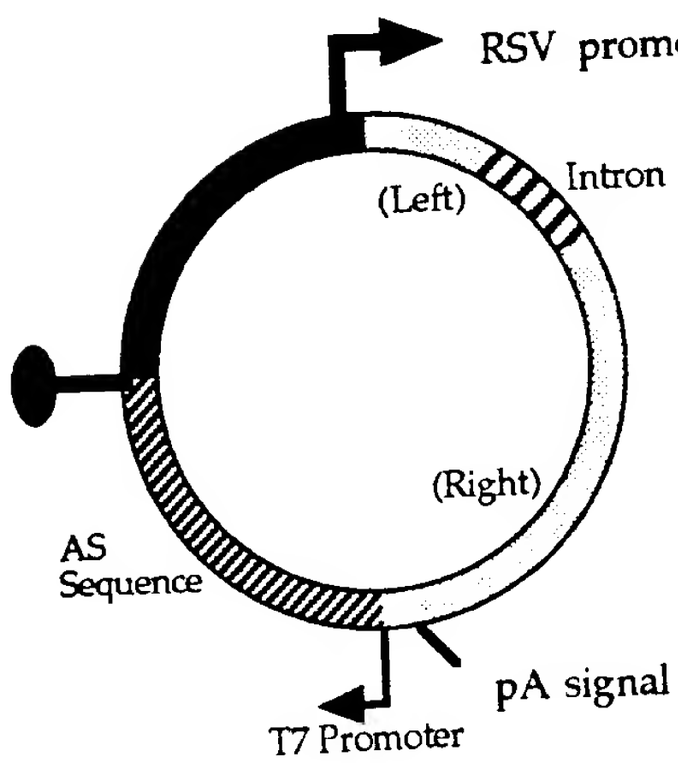
Fusion of PCR fragments together in eucaryotic expression vector

(B)



Introduction of cassette with AS directed from T7 promoter

(C)

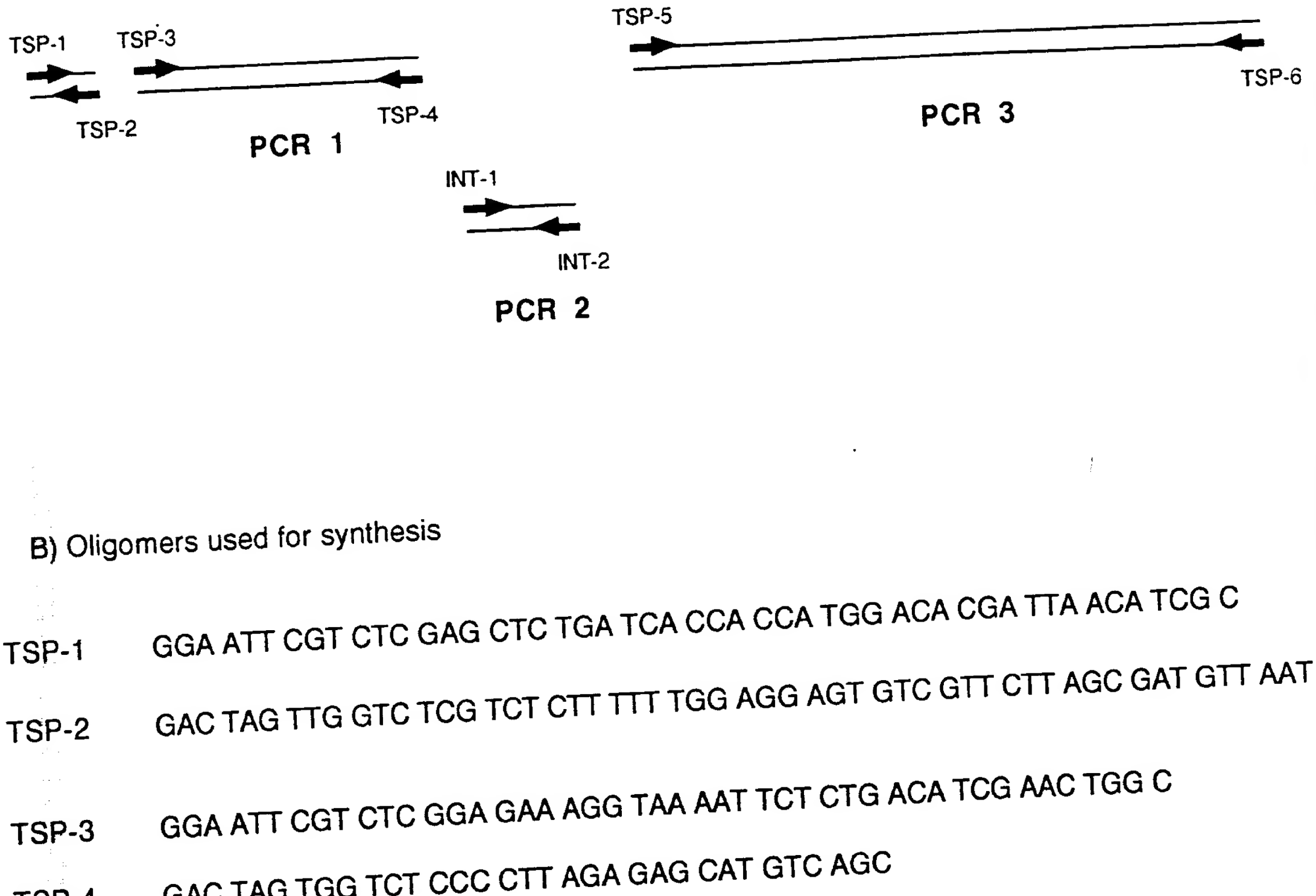


Active T7 RNA polymerase is only made in eucaryotic cells after splicing out of SV40 Intron

Figure 25

Construction of T7 Expression Vector

A) Synthesis of pieces



B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP-3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

Figure 26

Synthesis of Pieces for Construction of T7 RNA Polymerase with Intron

Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to Clone with PCR #1 product

Annealing of TSP1 with TSP2

TSP1

```
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GC    3'
                                   3' C TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG  5'
                                                                                                        TSP2
```

Extension of TSP1/TSP2 by polymerase

```
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC  3'
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG  5'
                                                                                  Bsa I
```

Digestion of TSP1/TSP2 product with Bsa I

```
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AA
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT
```

Digestion of PCR #1 clone (pL-1) with BsmB I

```
          Bsm BI
5' GGA ATT CGT CTC G          GAGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC---------
   CCT TAA GCA GAG CCTCT           TTC CAT TTT AAG AGA CTG TAG CTT GAC CG---------
```

Ligation of Bsa I digested TS1/TS2 product to BsmB I digested PCR#1 clone

```
5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG  AGA  AAG  GTA  AAA  TTC
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC  TCT  TTC  CAT  TTT  AAG

TCT  GAC  ATC  GAA  CTG  GC---------
AGA  CTG  TAG  CTT  GAC  CG---------
```

Figure 27

Comparison of the 5' ends of the Nucleotide Sequences of Wild Type and Modified T7 RNA Polymerase

Wild Type T7 nucleic and amino acid sequence

```
ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC TTC TCT GAC ATC GAA CTG GC----------
TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG AAG AGA CTG TAG CTT GAC CG----------
 1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16
```

Modified T7 nucleic and amino acid sequence with Nuclear Localisation Signal (NLS) insertion

```
ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC----------
TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT TTC CAT TTT AAG AGA CTG TAG CTT GAC CG----------
 1   2   3   4   5   6   7   8   9  10                                      11  12  13  14  15  16
```

Figure 28

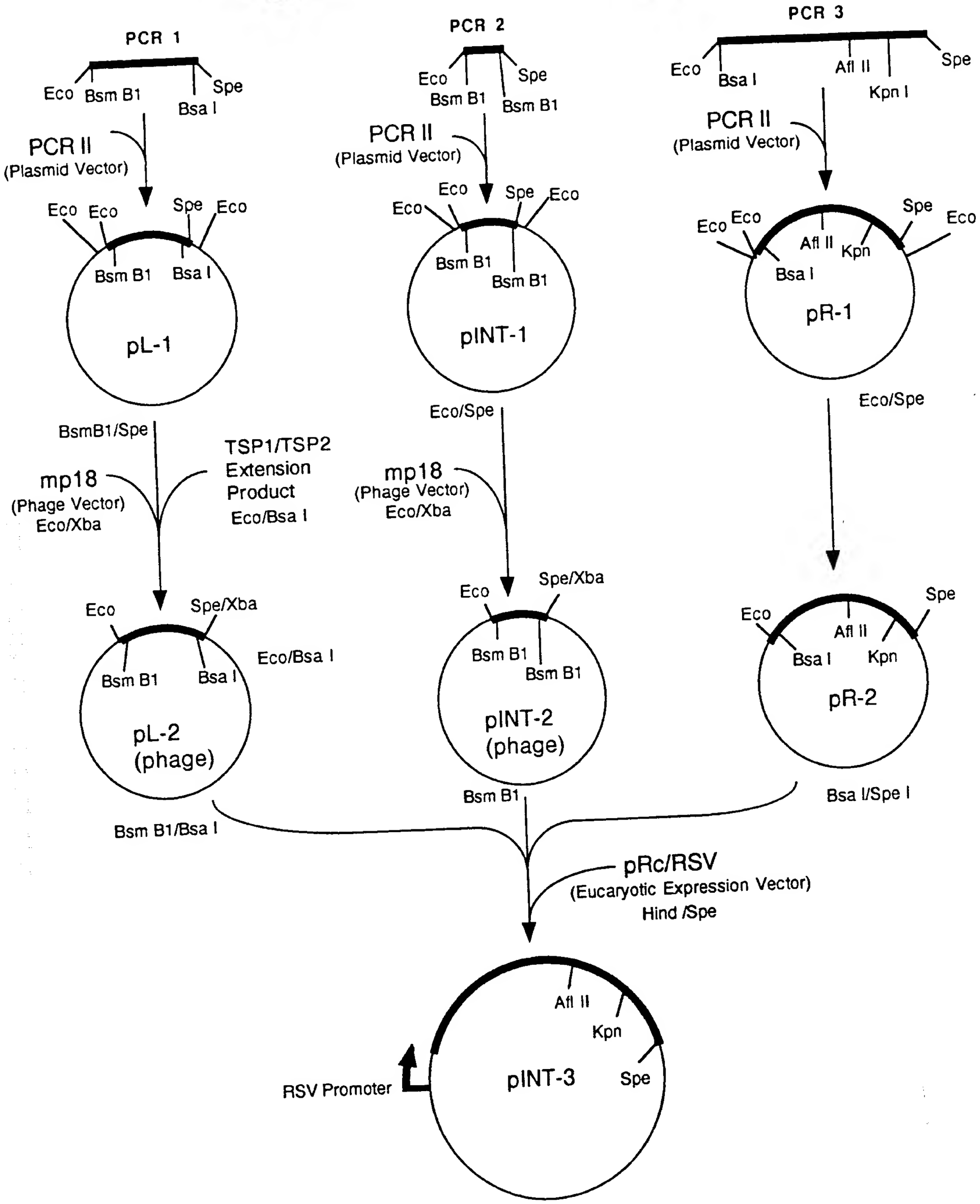


Figure 29

Fusion of PCR Pieces to Construct T7 RNA Polymerase with an Intron

(A) Oligomers

HTA-1	GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGCTTA AGC CTC AAG
HTA-2	GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
HTB-1	GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
HTB-2	GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC TTC CTG CCA TAG GAG AGC CTA AGG T
HTC-1	GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC AG
HTC-2	GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
TER-1	AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
TER-2	TTC TGC AGA TAT AGT TCC TCC TTT CAG C

(B) Cloning of AS and Terminator sequences into vector with T7 Promoter

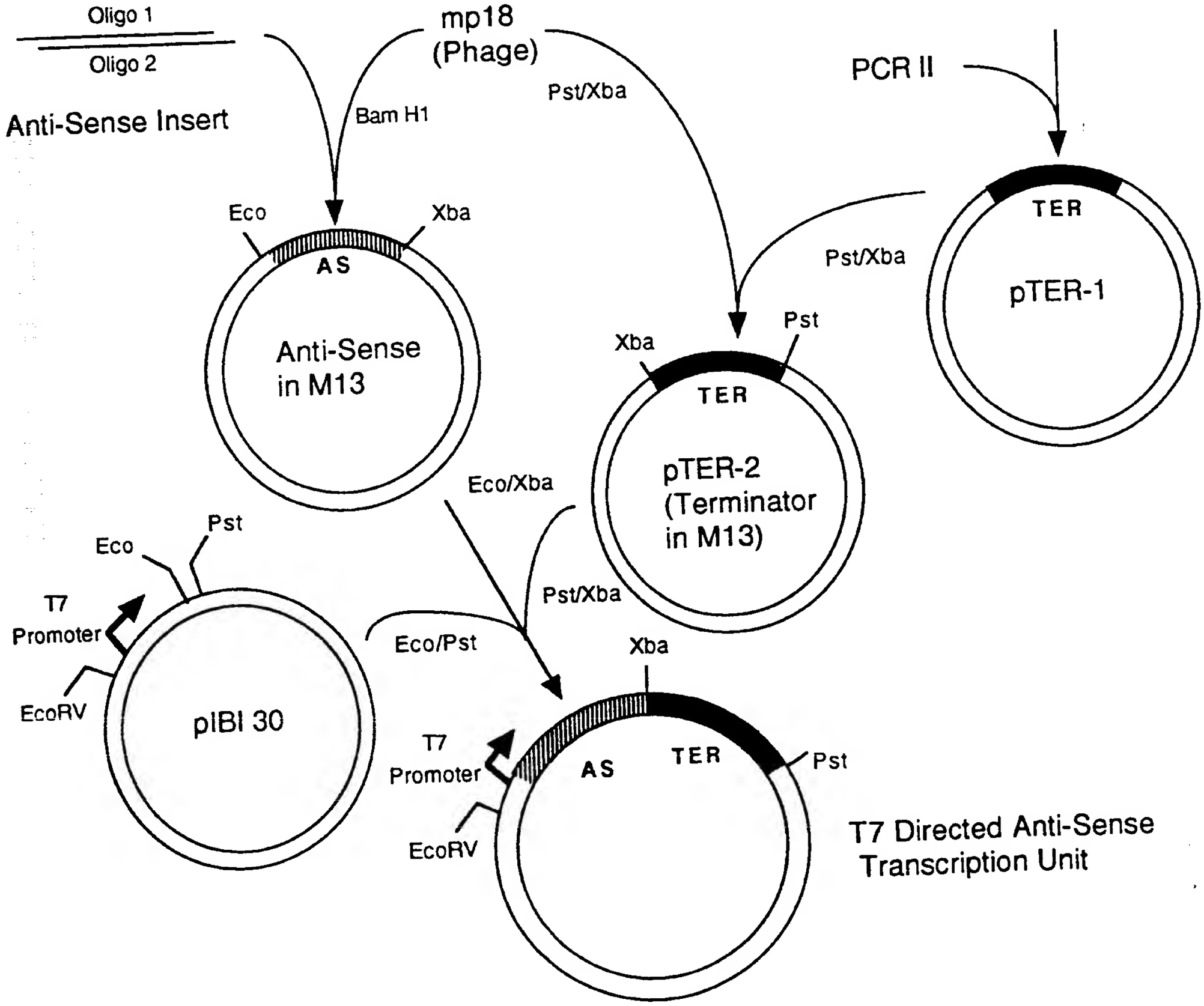
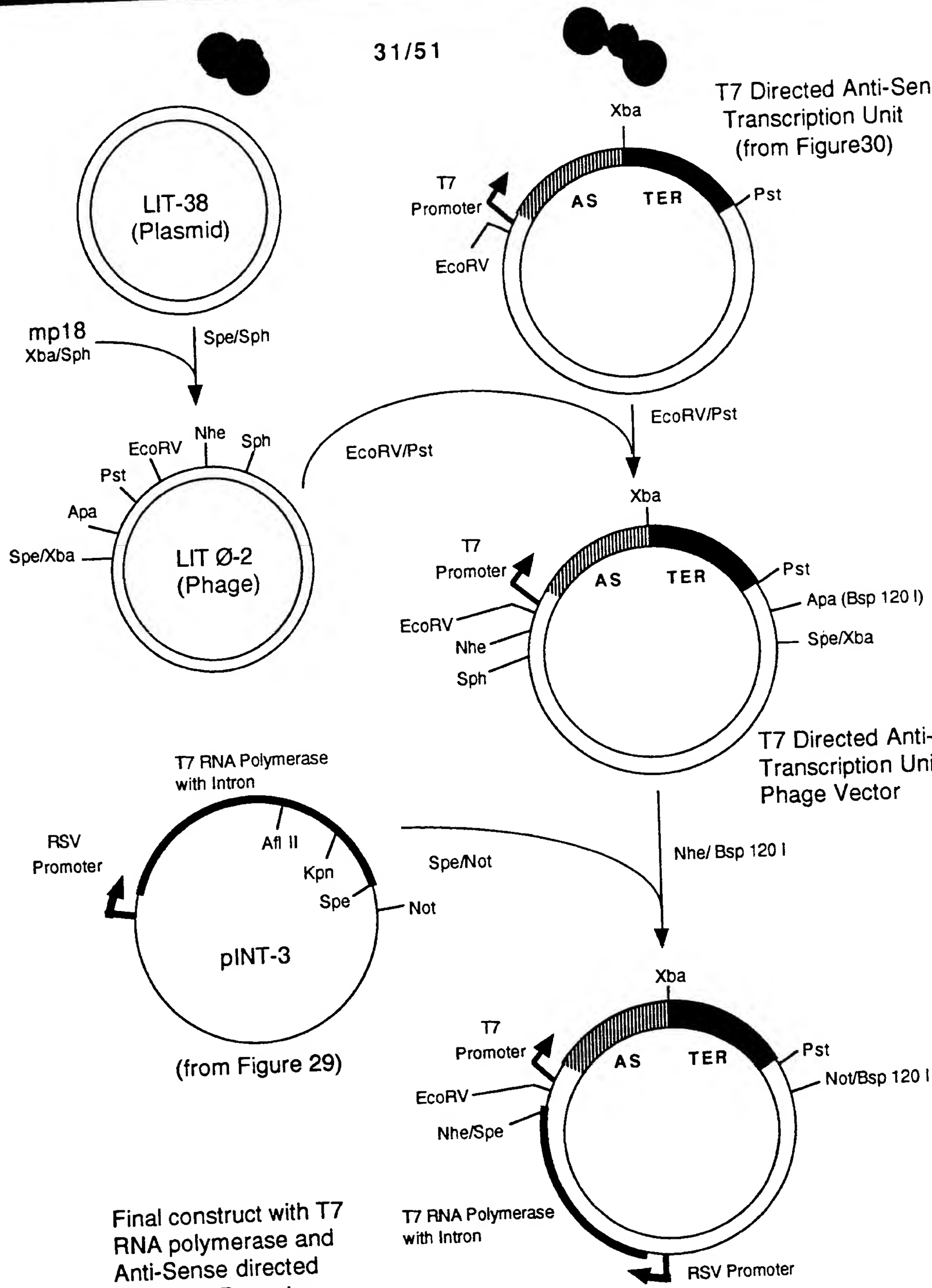


Figure 30

Insertion of Anti-Sense Sequences into T7 Directed Transcription Units



Final construct with T7 RNA polymerase and Anti-Sense directed from a T7 Promoter

Figure 31

Construct with T7 RNA polymerase and Anti-Sense directed from a T7 Promoter

A) Oligomers for introduction of T7 signals and polylinker

PL-1 TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT

AGT TAA ATG CAG ATC T

PL-2 CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG

ATC CTT AAG CCA TGG C

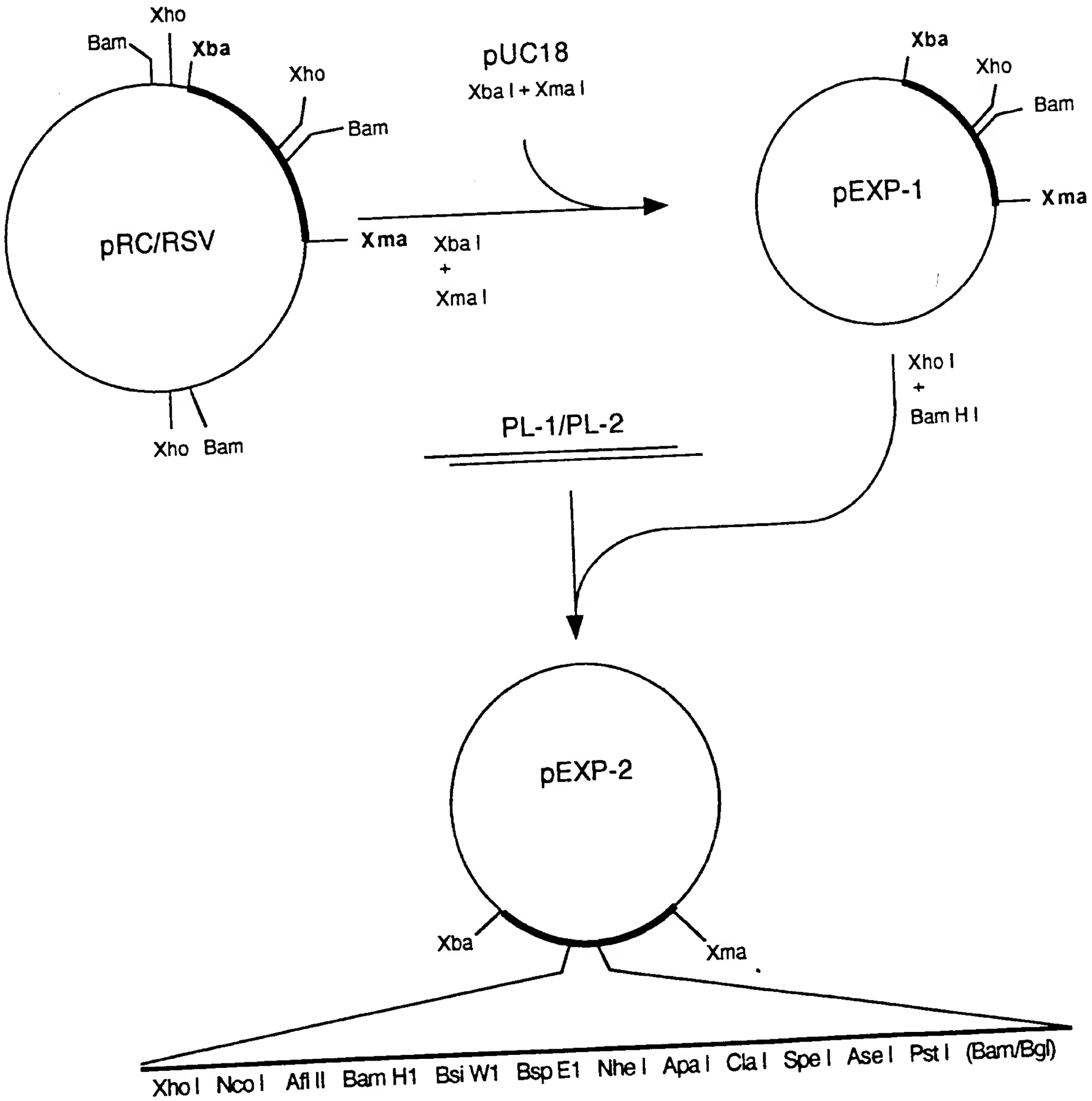
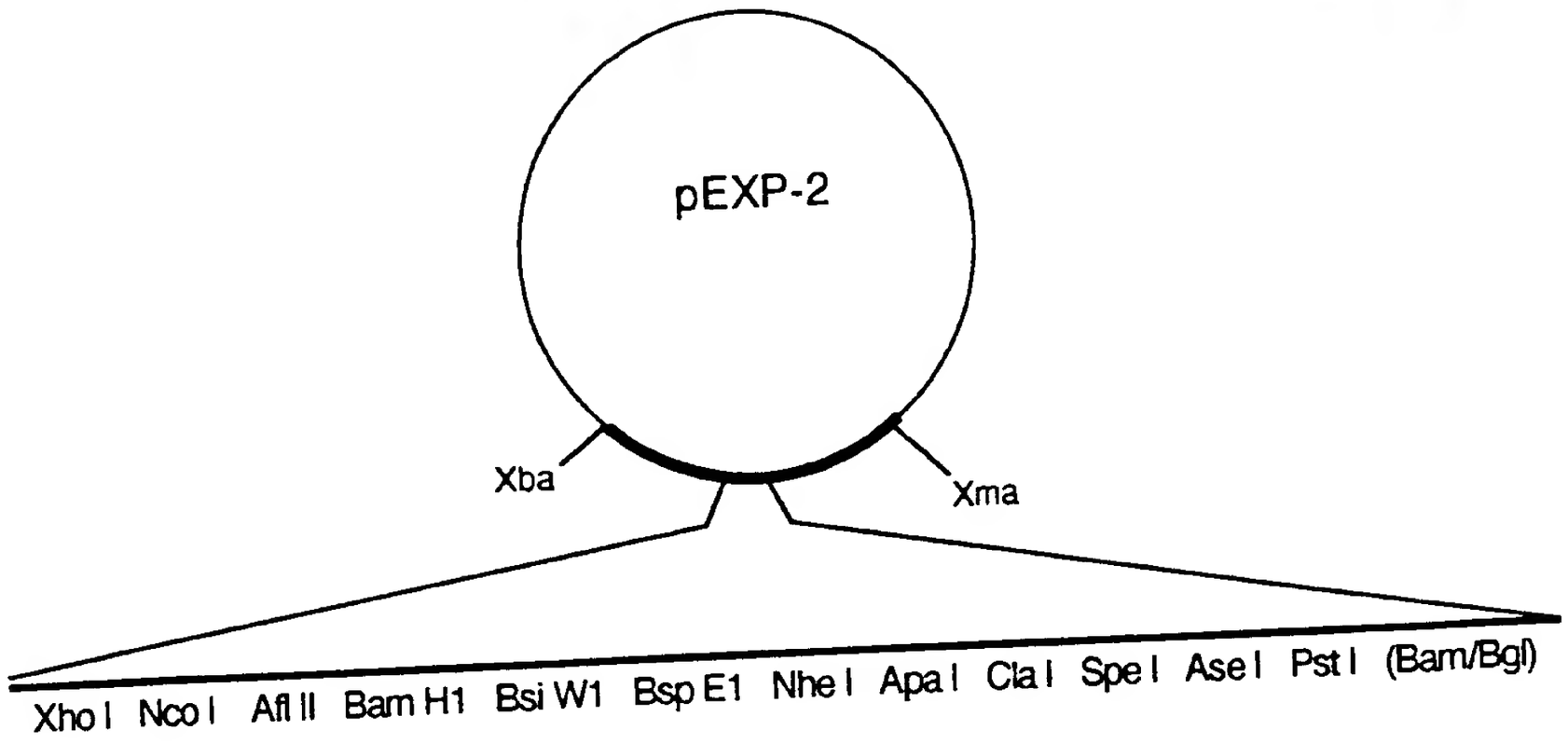


Figure 32

Introduction of Poly-Linker for Creation of Protein Expression Vector



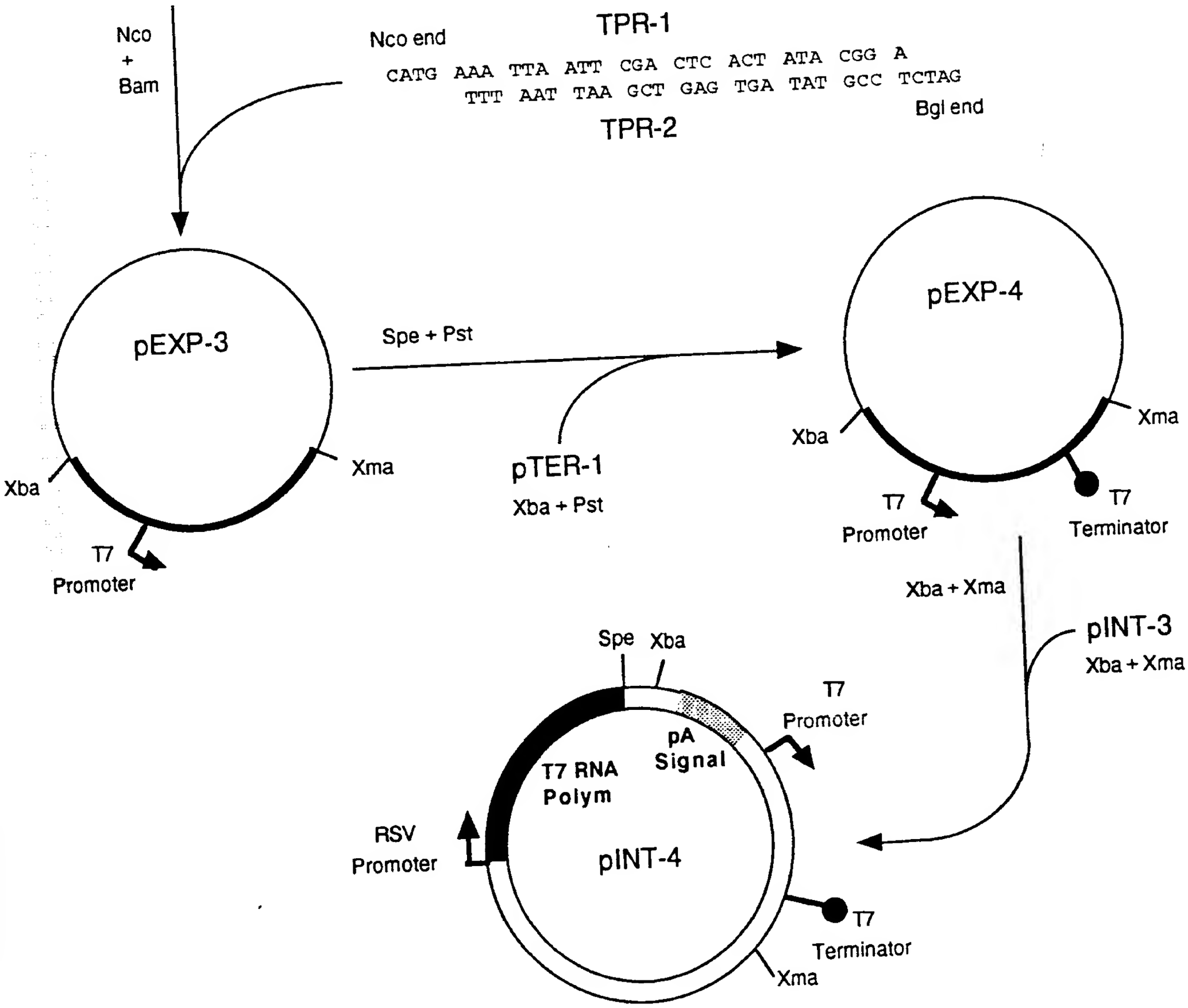


Figure 33

Final steps for construction of Expression Vector

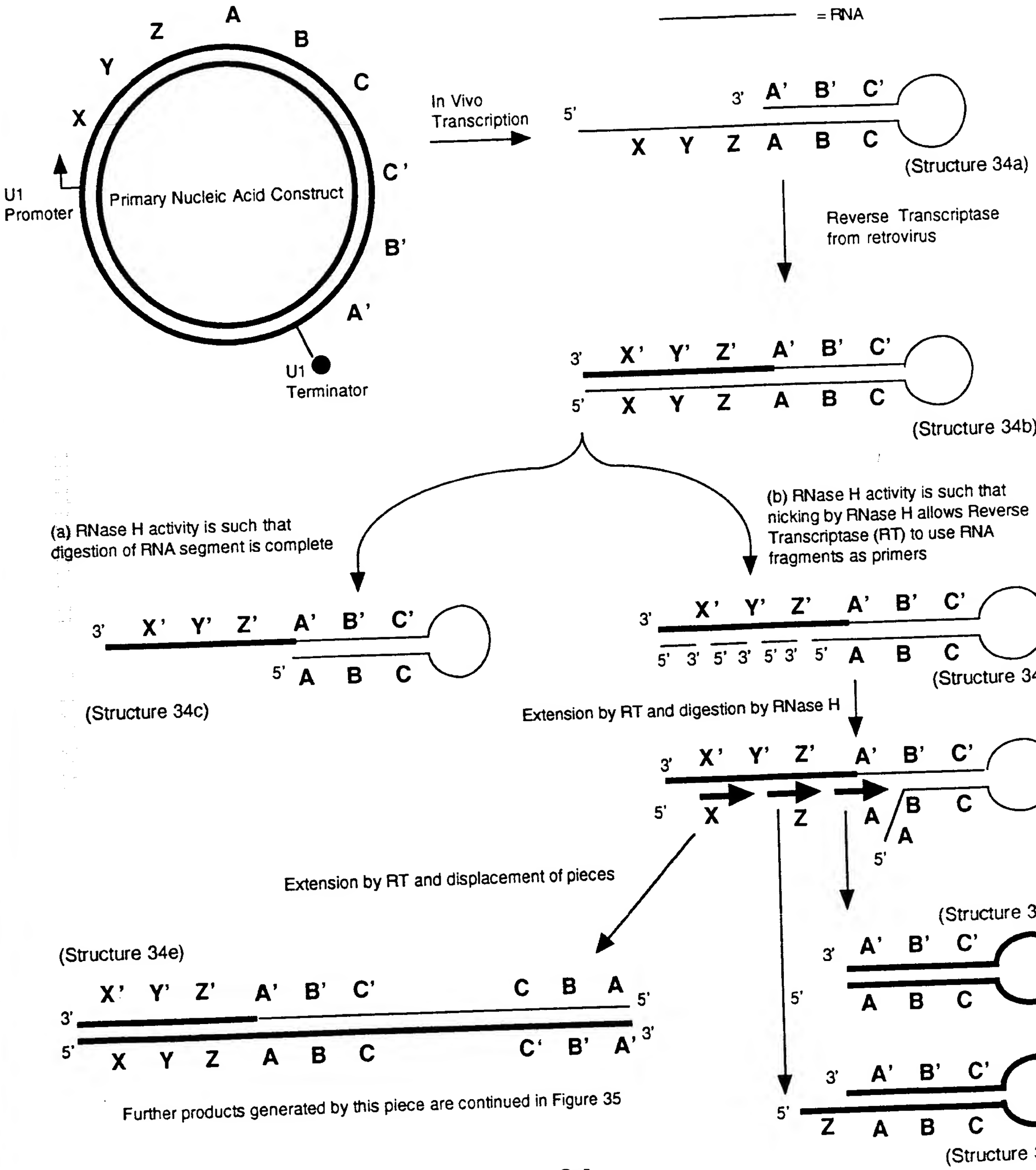


Figure 34

Construct that produces single-stranded Anti-Sense DNA

Continued from Figure 34

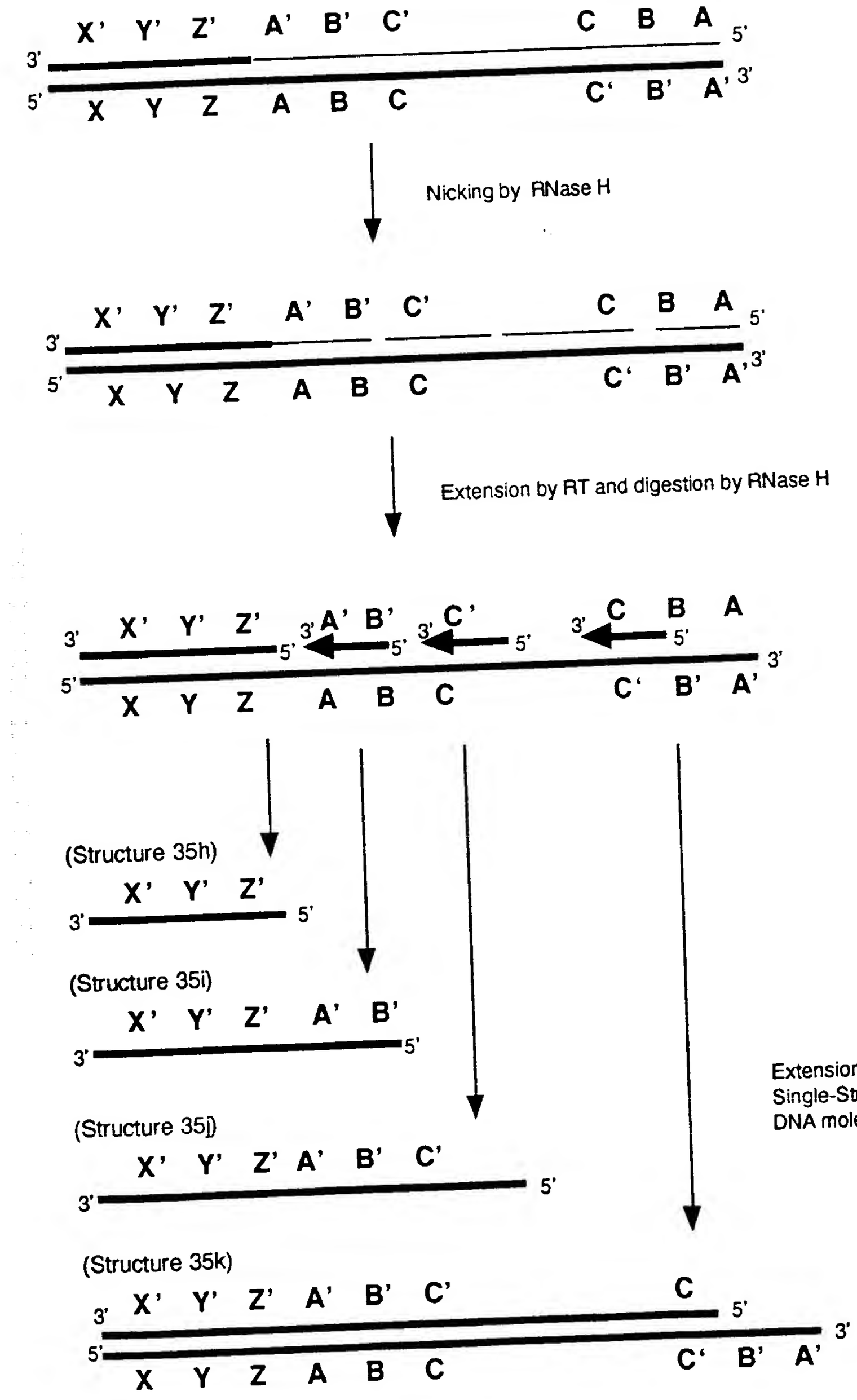
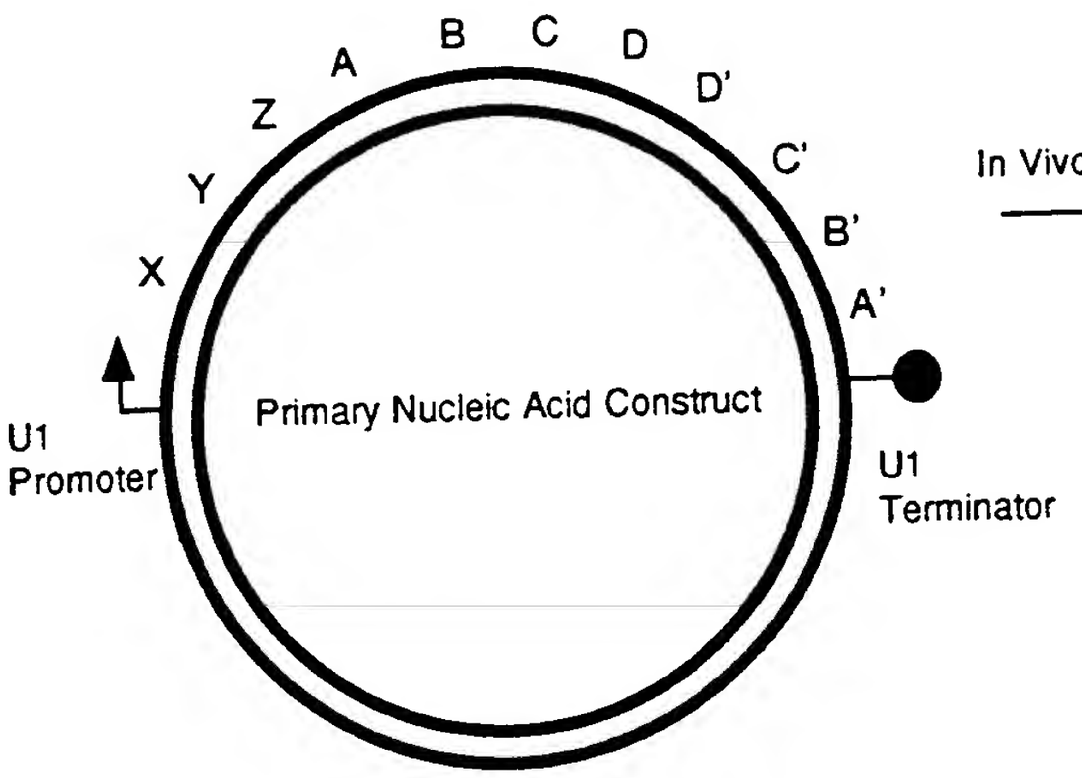
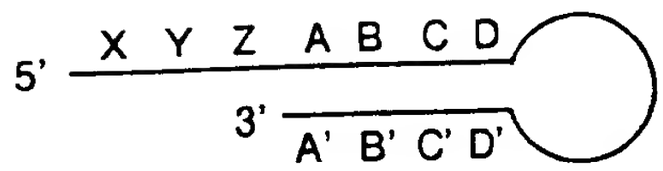


Figure 35
Continuation of Process from Figure 34



In Vivo Transcription

(Structure 36a)

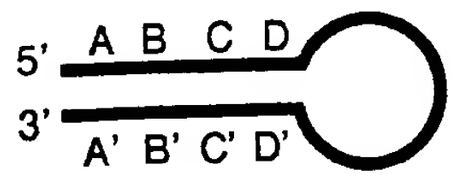


In a series of events similar to that shown for Example G-1, the net products of Rnase H and RT activities on the transcript above create Double-stranded DNA products similar to these below:

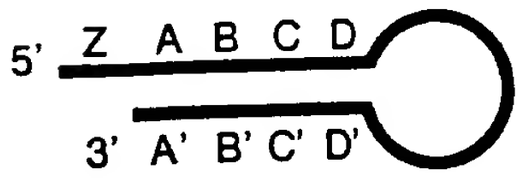
= DNA

= RNA

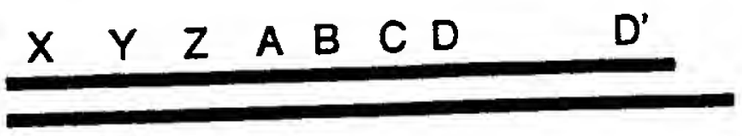
(Structure 36b)



+ (Structure 36c)



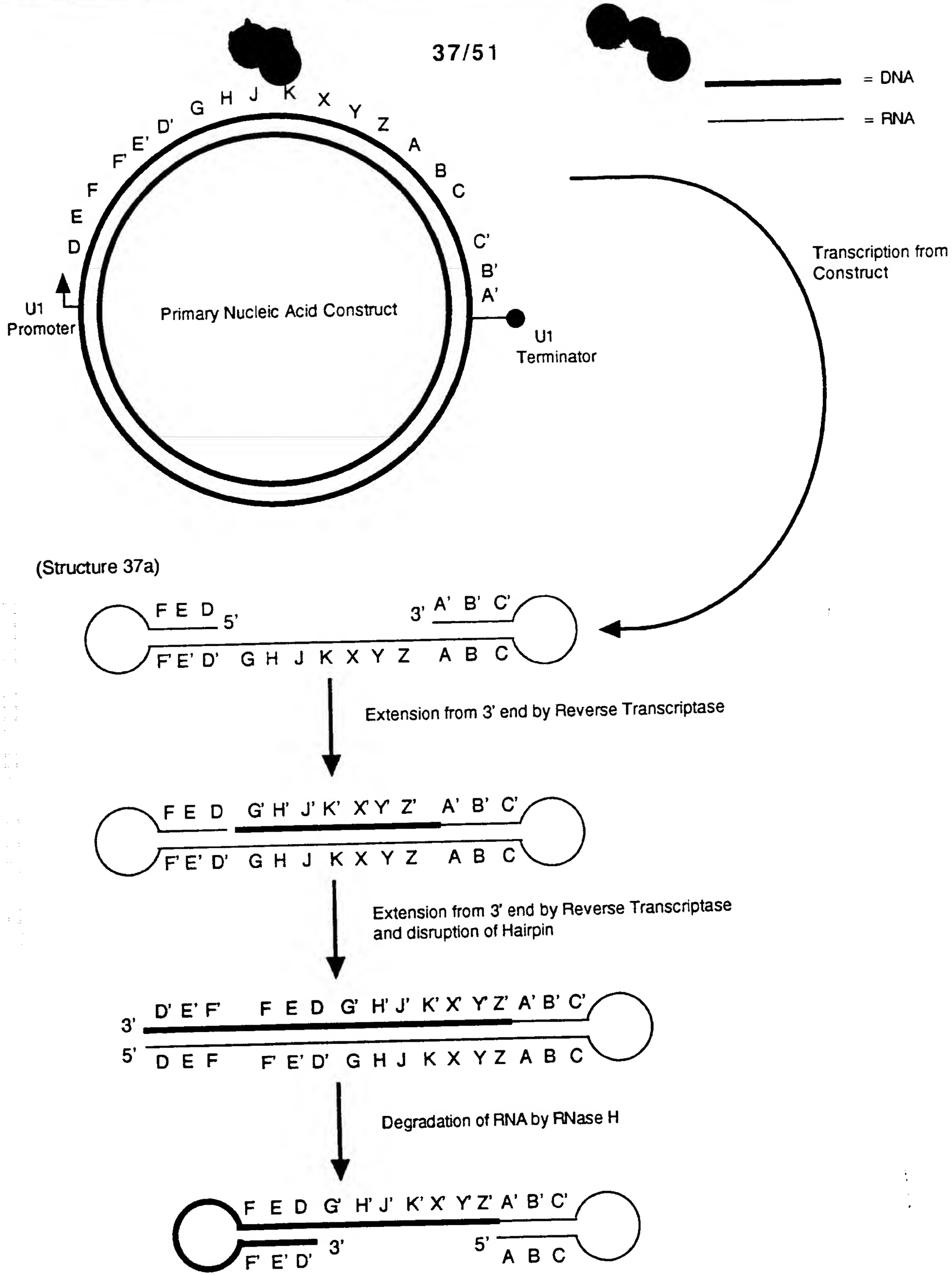
+ (Structure 36d)



In this example, A B C is a promoter sequence, directing transcription off of these Double-stranded DNA products to create RNA transcripts with varying amounts of double-stranded character. Furthermore, the single-stranded loop segment (D to D') of the transcript codes for anti-sense sequences

Figure 36

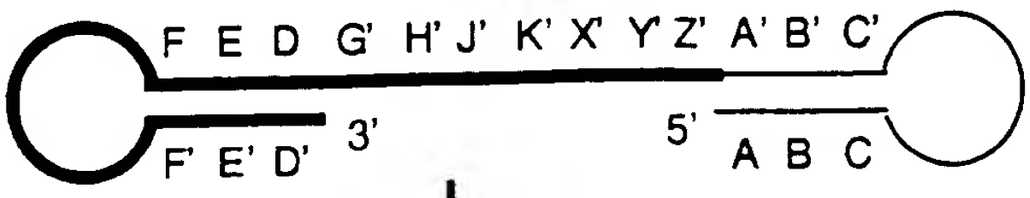
Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription



(Continued in Figure 38)

Figure 37

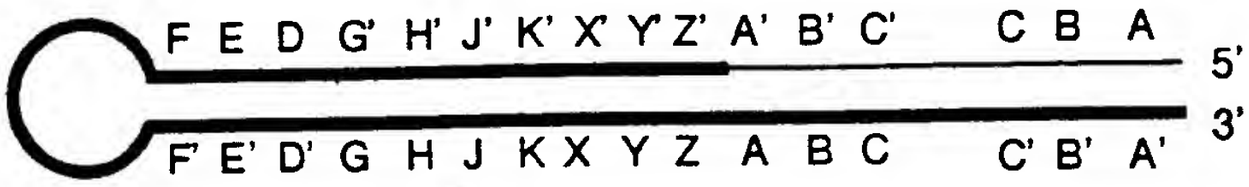
Construct which Propagates a Double Hairpin Production Center



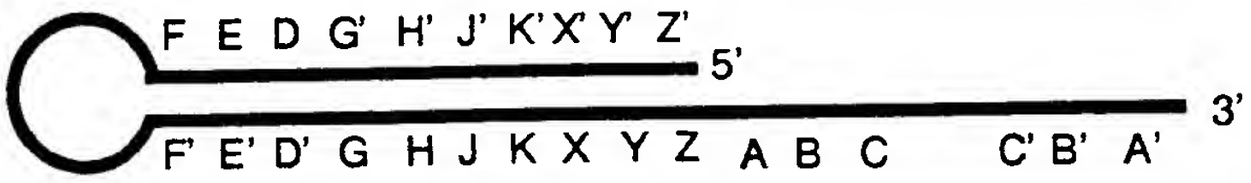
= DNA

= RNA

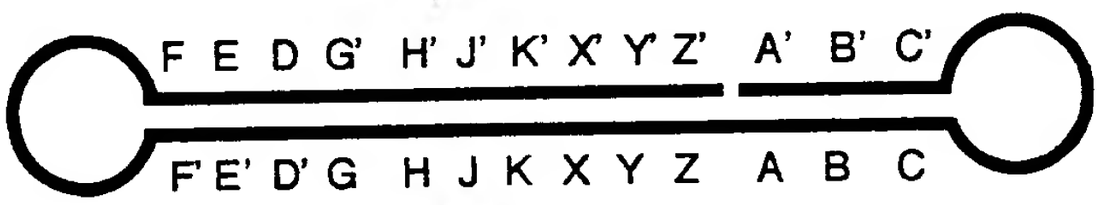
Extension from 3' end by Reverse Transcriptase



Degradation of RNA by RNase H



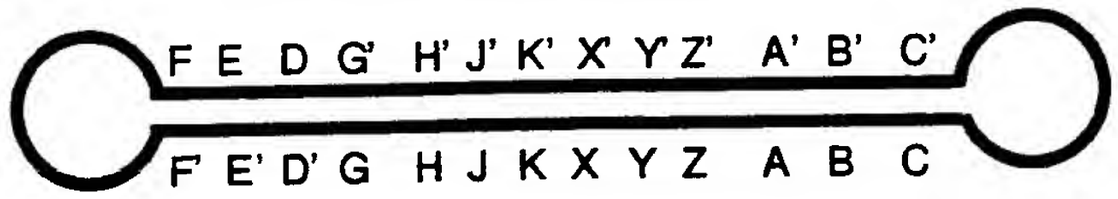
Formation of hairpin



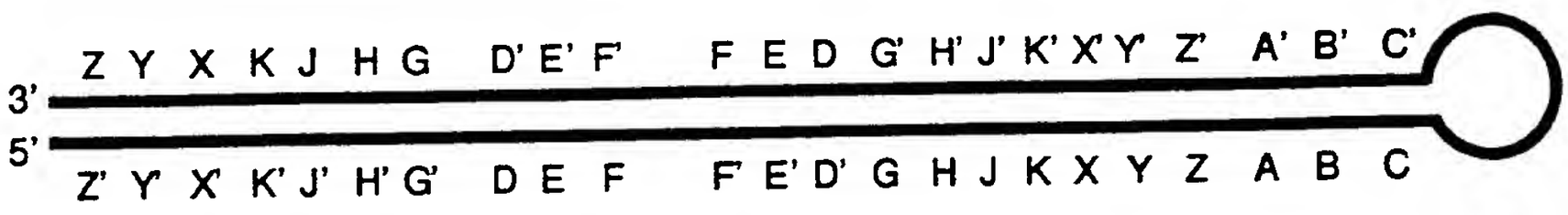
(Structure 38b)

(A) Sealing of nick by DNA Ligase

(B) Displacement at nick and further extension



(Structure 38c)

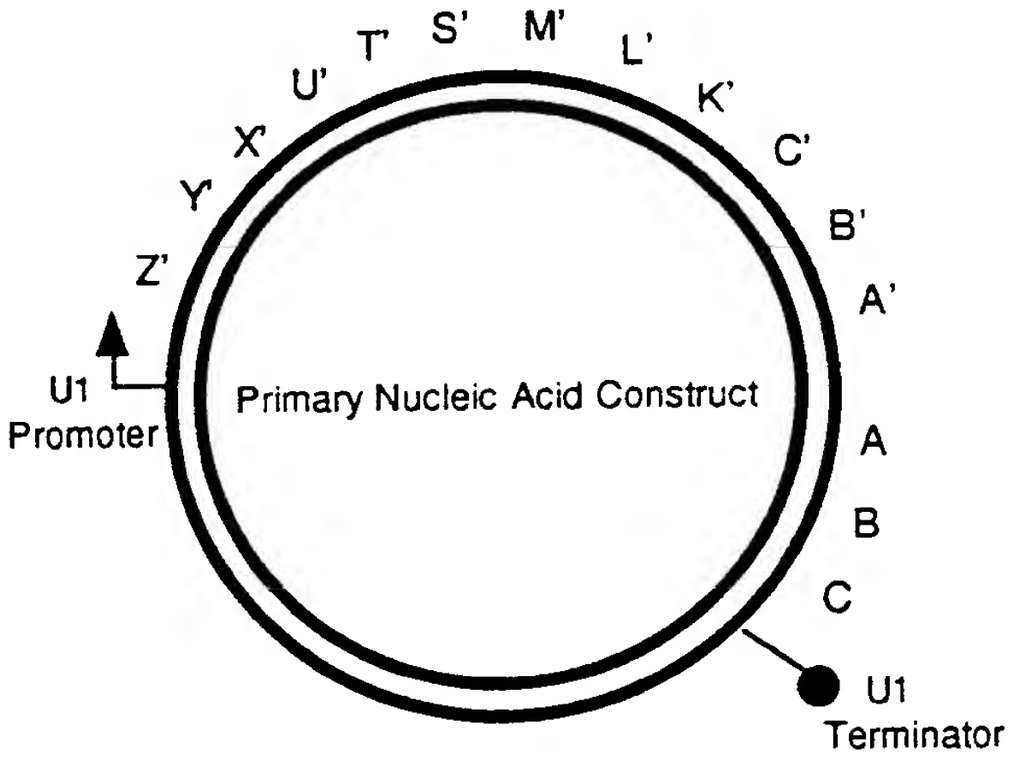


(Structure 38d)

In this Example, the sequence F' E' D' is a promoter, the sequence G H J K is an Anti-Sense sequence and X Y Z is a Poly A signal

Figure 38

Continuation of process from Figure 37



= DNA

= RNA

In vivo transcription

5' Z' Y' X' U' T' S' M' L' K' C' B' A'
3' C B A

(Structure 39a)

Splicing out of Intron on Anti-coding strand. In this example the sequence X' U' T' S' M' reflects splice donor, intron and splice acceptor sequences.

5' Z' Y' X' M' L' K' C' B' A'
3' C B A

(Structure 39b)

A series of RNase H and Reverse Transcriptase steps as shown in Figures 34 and 35

5' Z' Y' X' M' L' K' C' B' A' A
3' Z Y X M L K C B A A'

(Structure 39c)

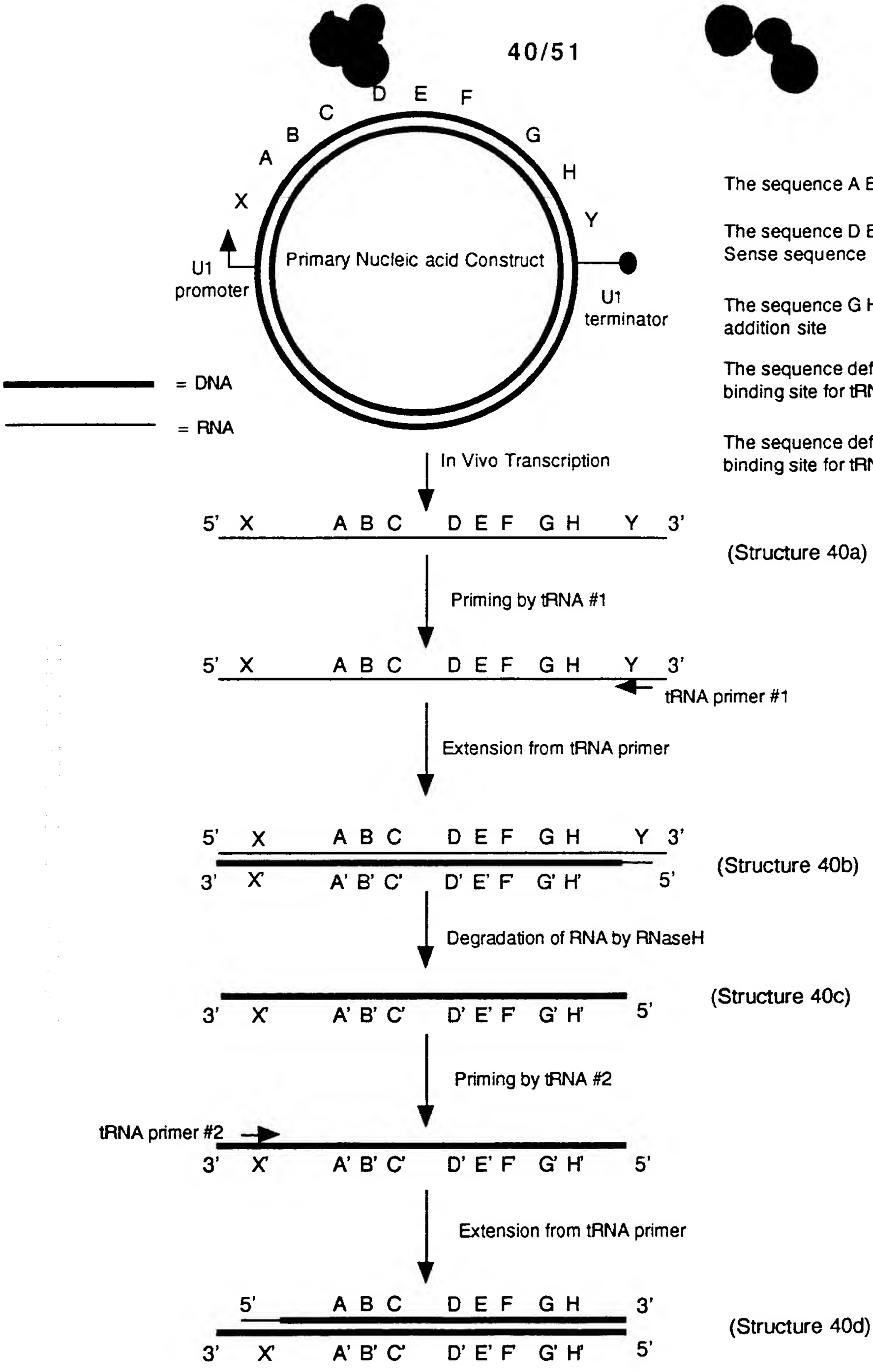
In vivo transcription

An X M L K 5' mRNA for diptheria toxin

In this Example:
A B C defines the sequence of an HIV LTR,
K L M X defines the sequence of diptheria toxin
YZ defines the sequence for a poly A signal.

Figure 39

Construct which propagates a Production Center capable of Inducible Suicide



The sequence A B C defines a promoter

The sequence D E F defines an Anti-Sense sequence

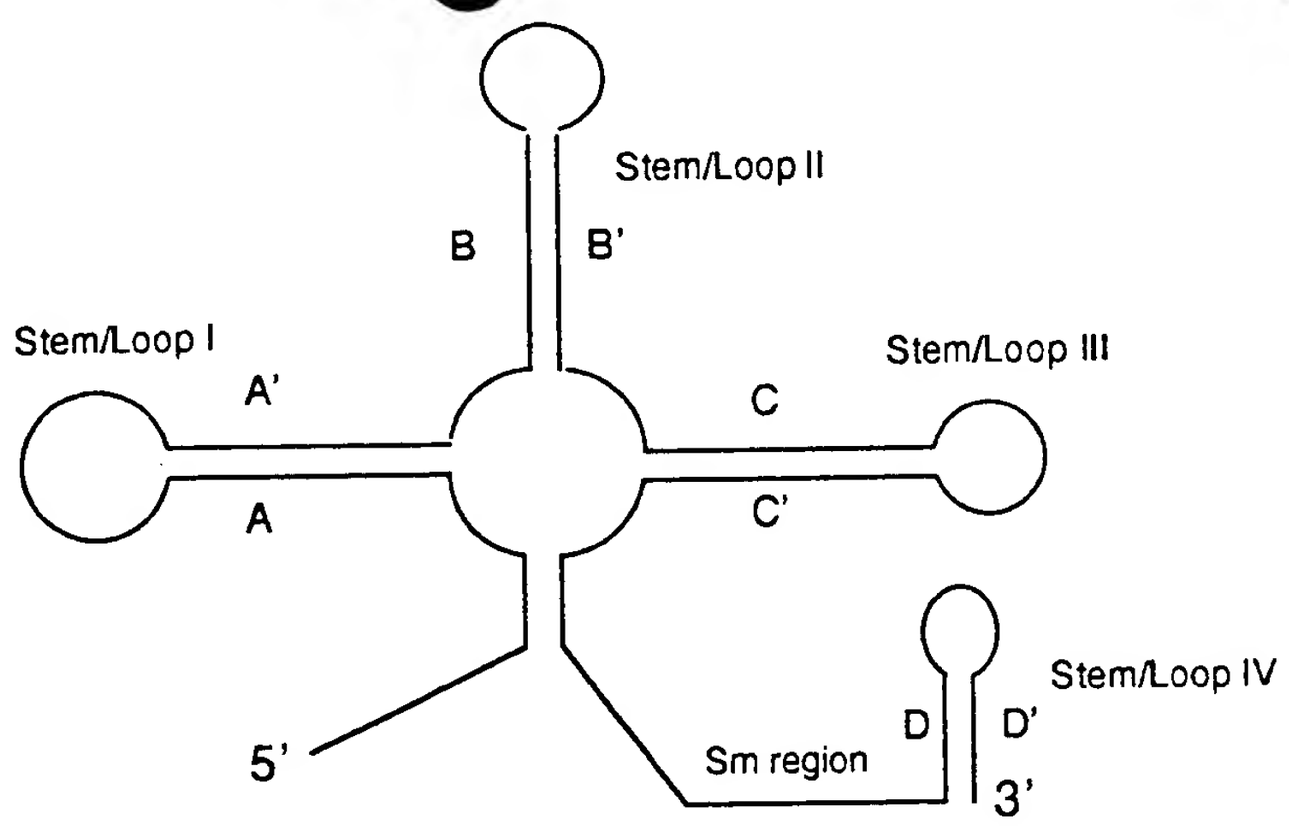
The sequence G H defines a poly A addition site

The sequence defined by Y defines a primer binding site for tRNA primer #1

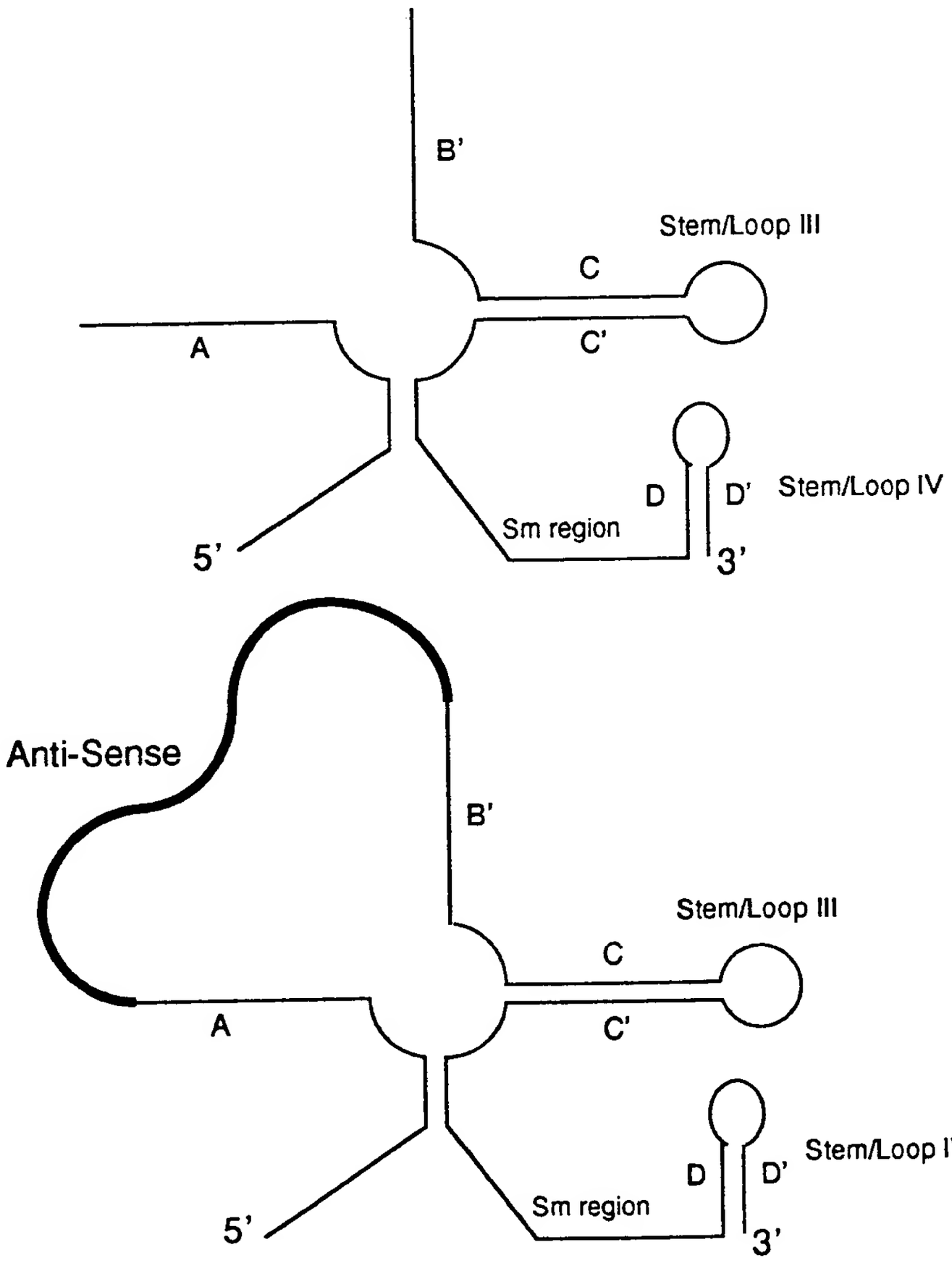
The sequence defined by X' defines a primer binding site for tRNA primer #2

Figure 40

Use of tRNA primers to create a DNA construct for secondary production of transcripts



Normal U1



U1 with
Bcl 1/Bsp EI
piece removed

U1 with Anti-Sense
sequence inserted

Figure 41

Excision of Sequences from U1 Transcript Region and Replacement with Novel Sequences

(A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT

HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG

HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT

HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG

HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT

HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG

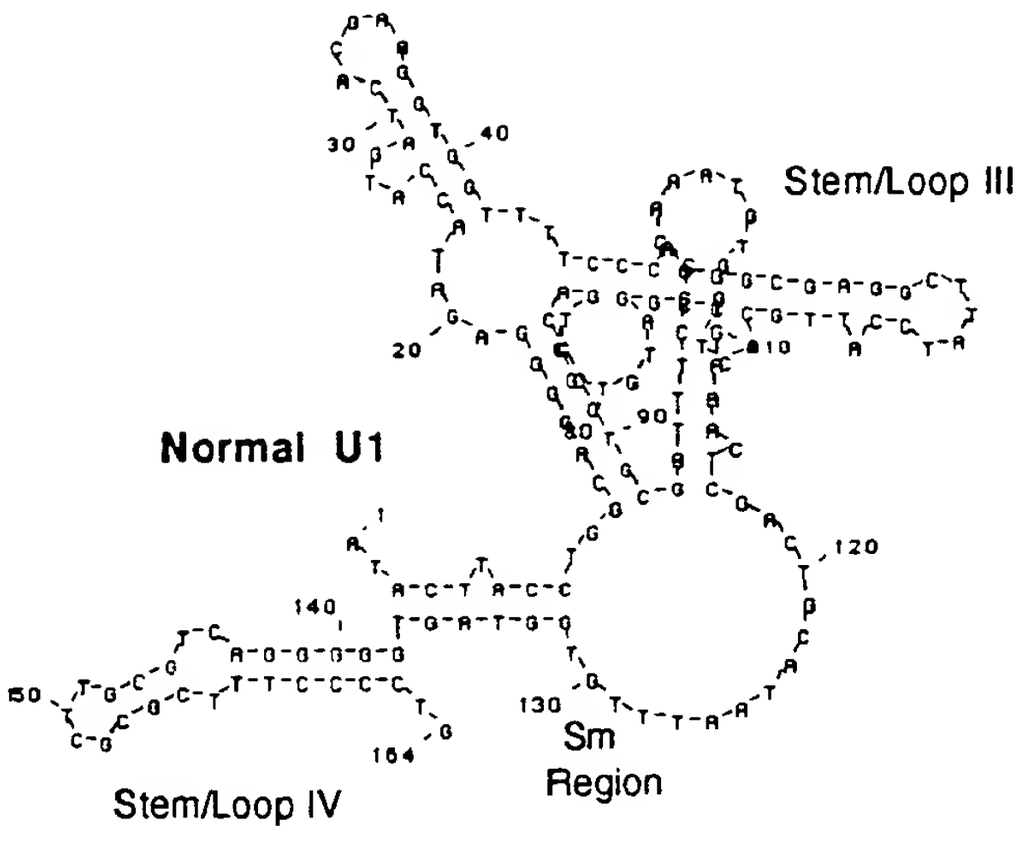
HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GT C GTA TTA T

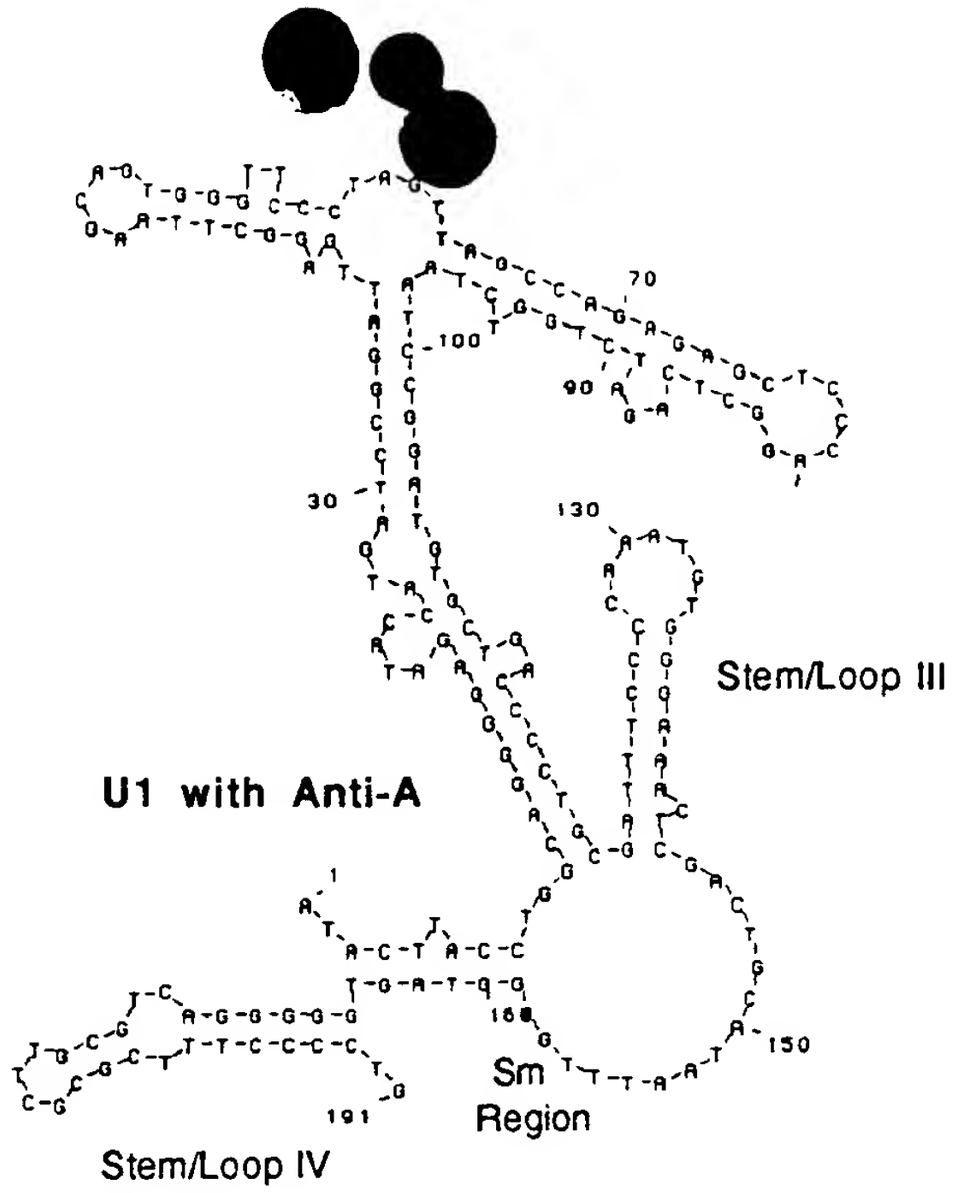
HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

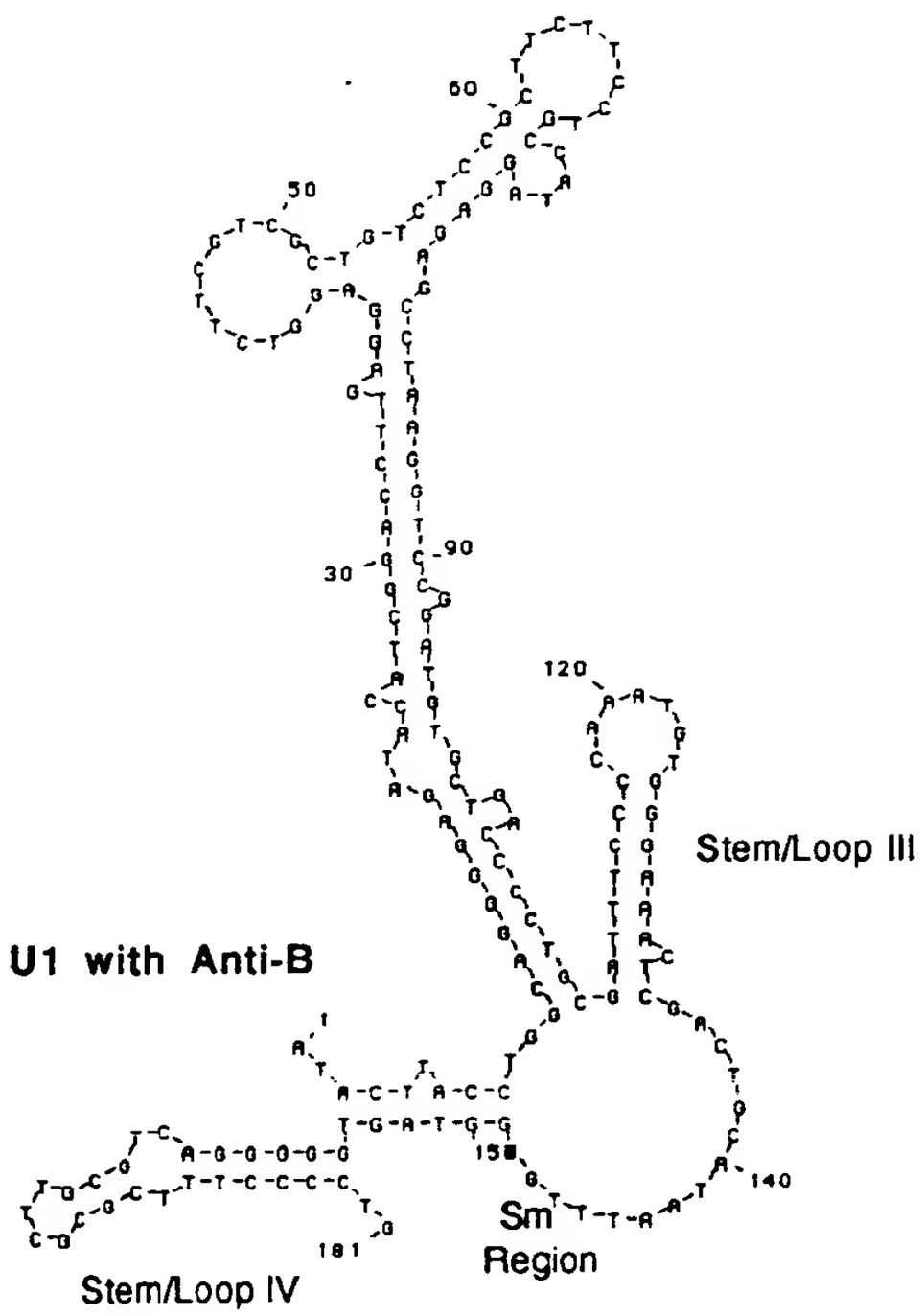
(B) Replacement of U1 sequences with HIV Anti-sense sequences

Figure 42

Insertion of Anti-Sense Sequences into U1Operons







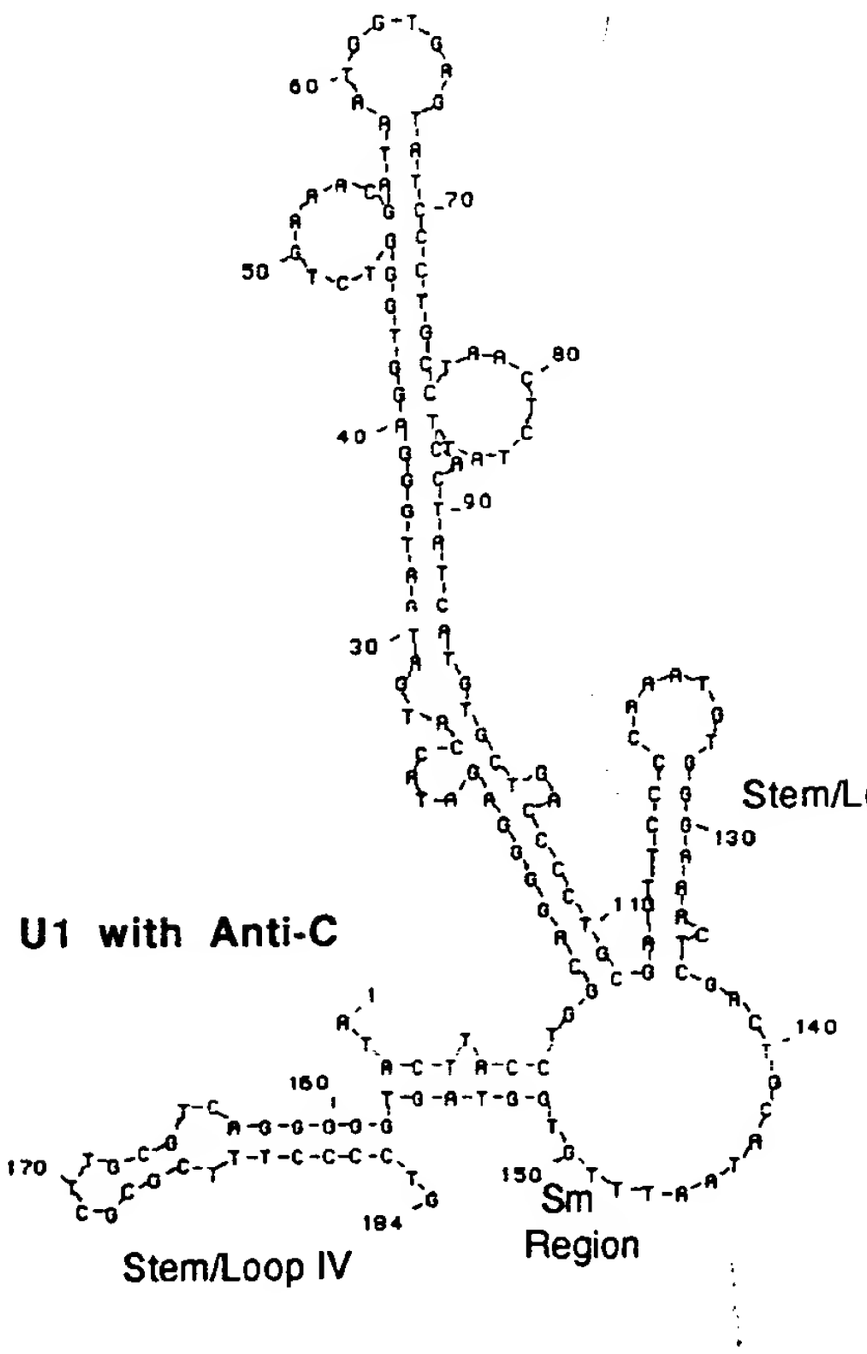


Figure 43

Predicted Secondary structures for U1 Transcripts with Anti-sense Substitutions

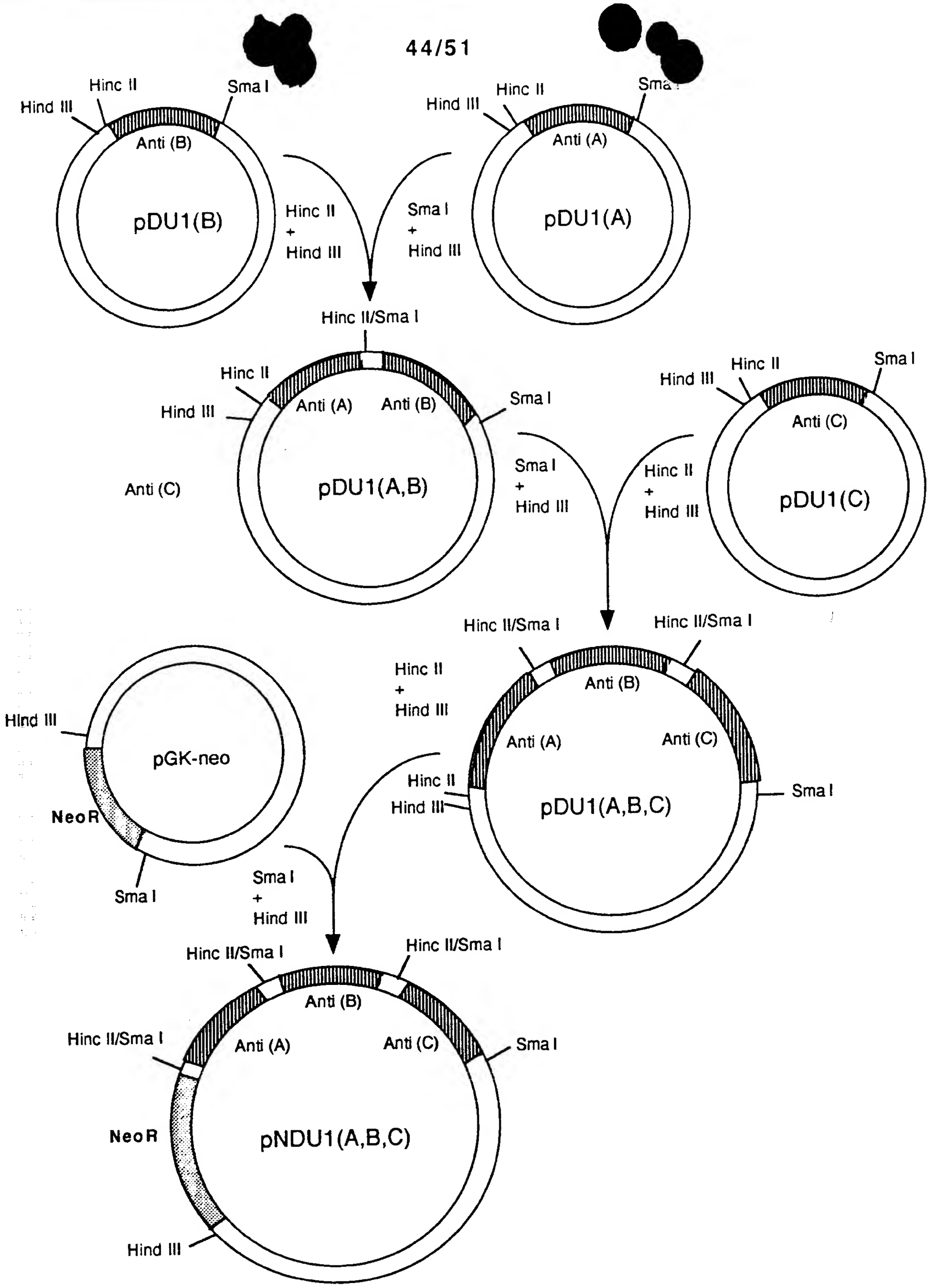


Figure 44

Construction of U1 Multiple Operon Clone

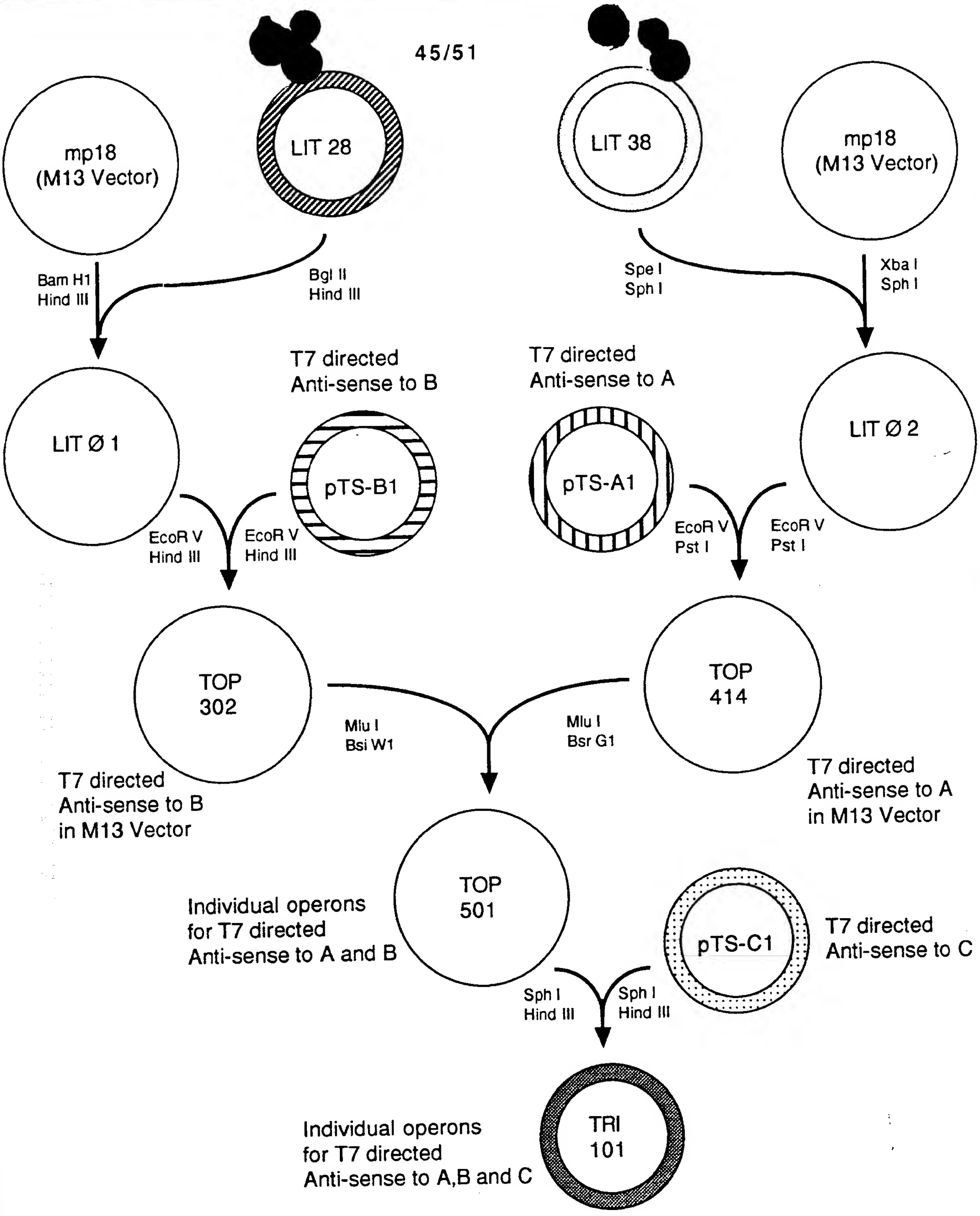
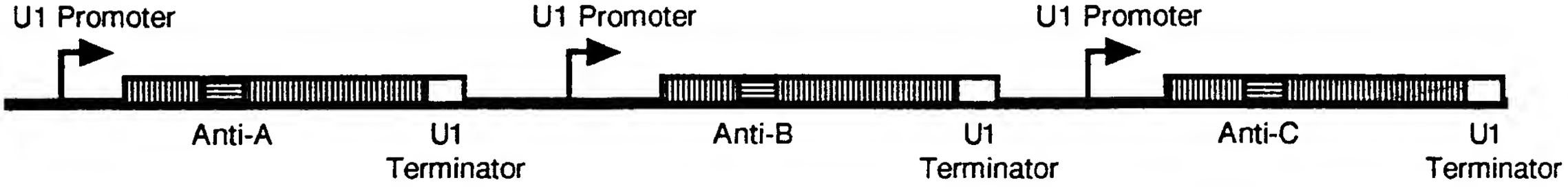


Figure 45

Construction of T7 Triple Operon

pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



TRI 101

Triple T7 Operon Construct with HIV Anti-Sense

Figure 46

Structures of Triple Operon Constructs
from Figures 44 and 45

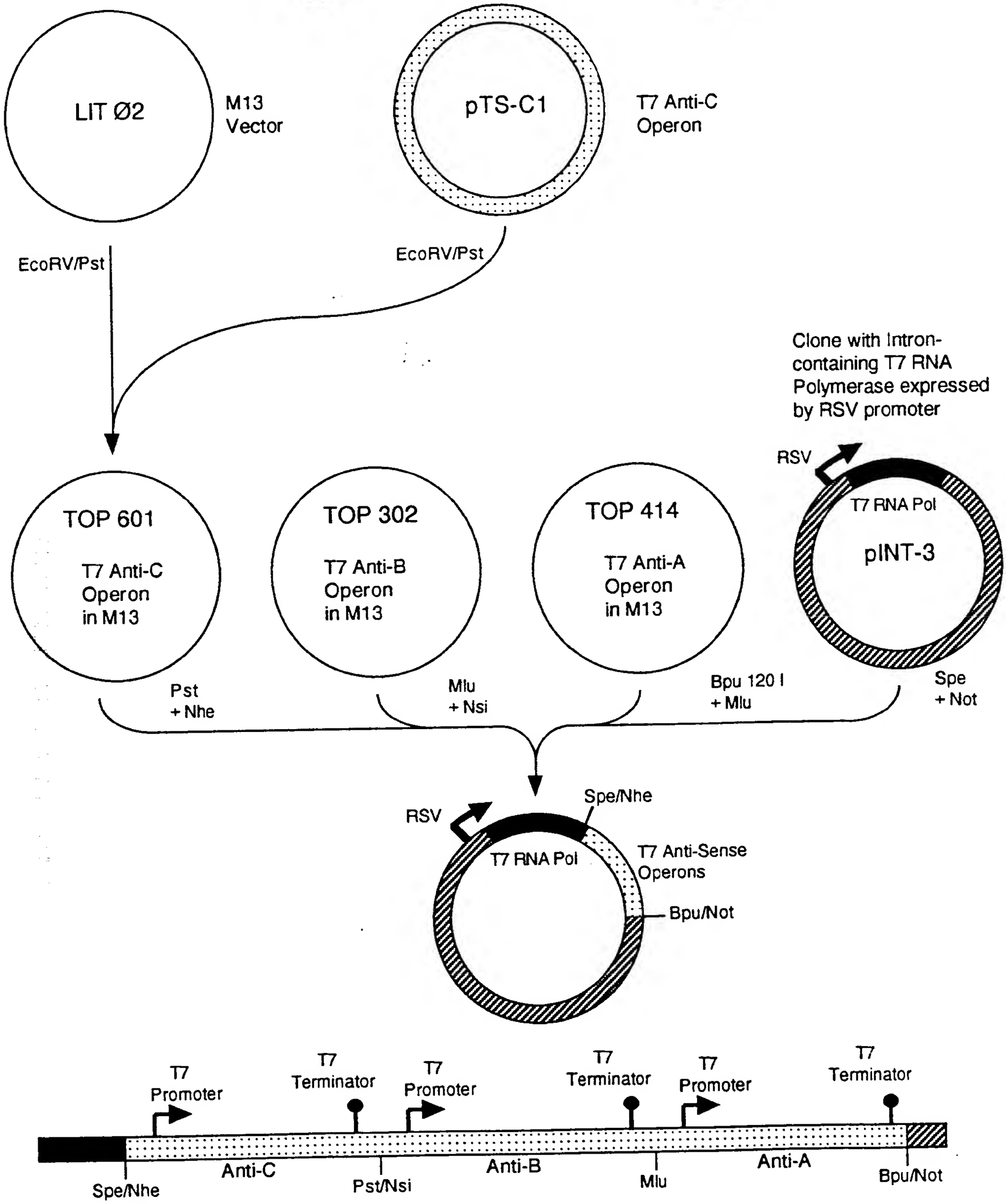
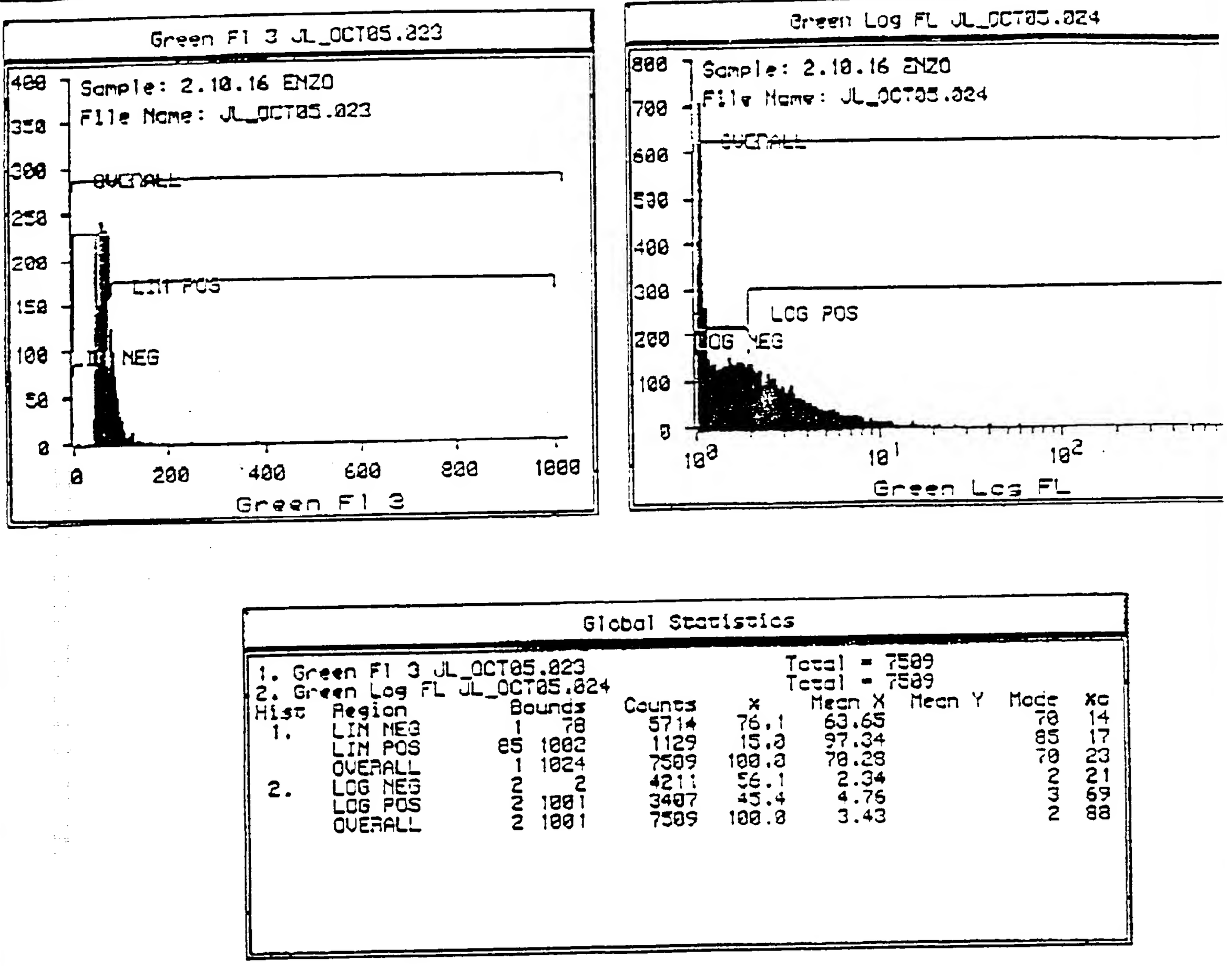


Figure 47

Construction of Multiple T7 Operons in Vector coding for T7 RNA Polymerse



Global Statistics

1. Green F1 3 JL_OCT05.023 Total = 7509
2. Green Log FL JL_OCT05.024 Total = 7509

Hist	Region	Bounds		Counts	%	Mean X	Mean Y	Mode	Xc
1.	LIM NEG	1	78	5714	76.1	63.65		78	14
	LIM POS	85	1002	1129	15.0	97.34		85	17
	OVERALL	1	1024	7509	100.0	78.28		78	23
2.	LOG NEG	2	2	4211	56.1	2.34		2	21
	LOG POS	2	1001	3407	45.4	4.76		3	69
	OVERALL	2	1001	7509	100.0	3.43		2	88

Figure 48

Flow cytometry data measuring binding of anti-CD4+ antibody to HIV resistant U037 cells

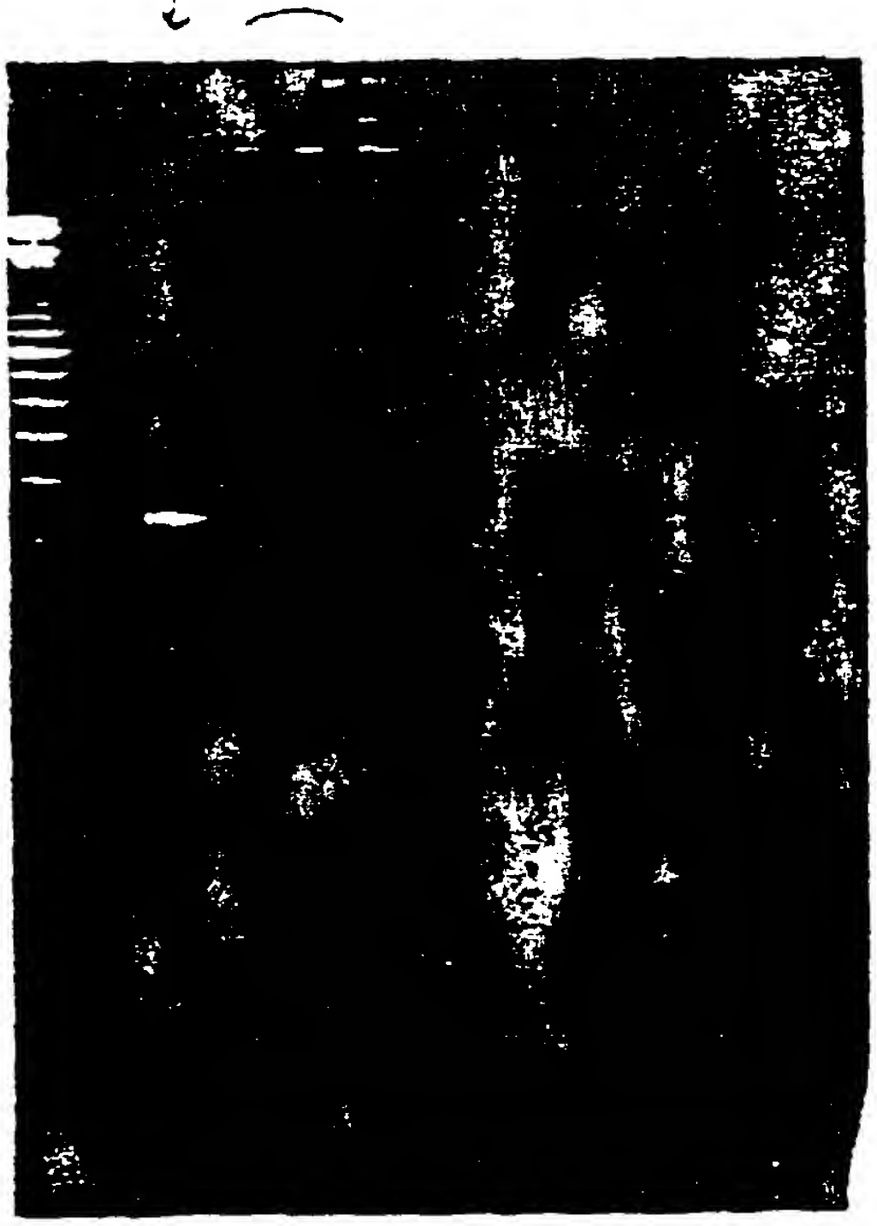


Figure 49

PCR amplification of gag region indicating absence of HIV in viral resistant cell line (2.10.16) after challenge

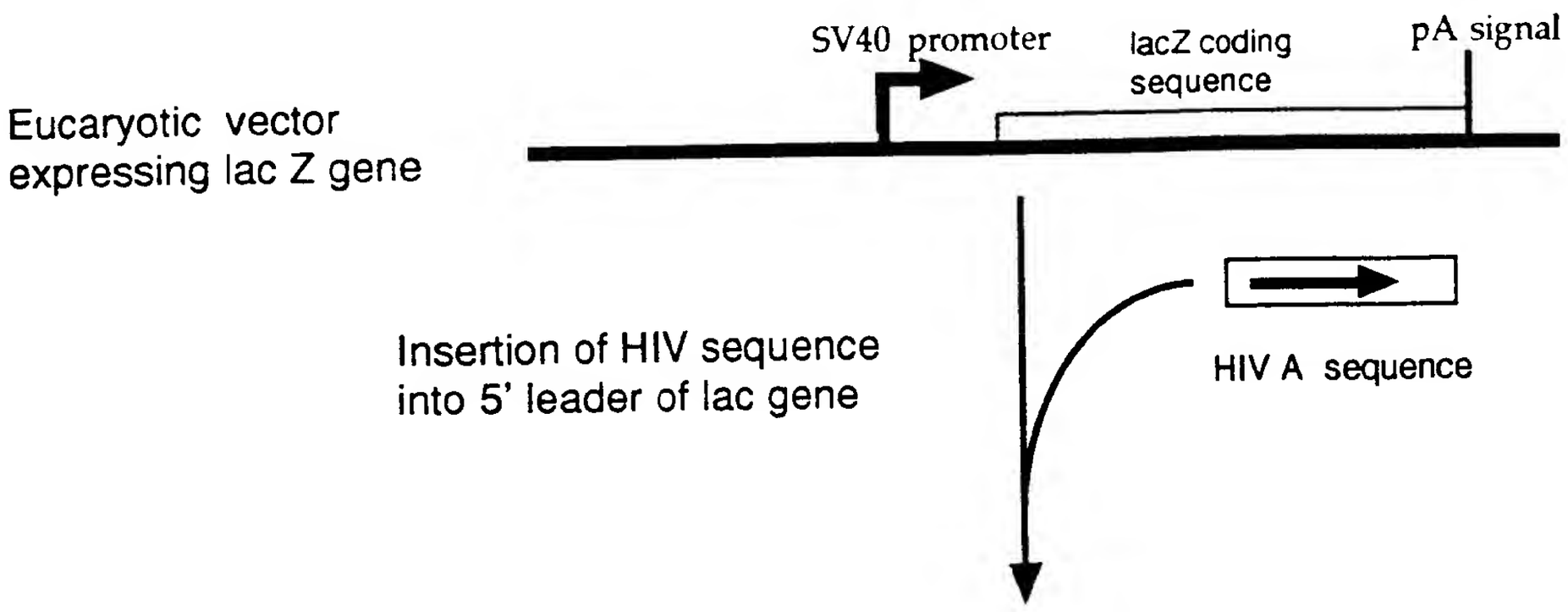


Figure 50

Clone with target-lacZ fusion will have reduced expression of lacZ after transfection by HIV Anti-sense construct

	Enzyme activity as expressed by A_{420} readings in extracts prepared from		
	2.5 x 10^4 cells	5 x 10^4 cells	1.0 x 10^5 cells
U 937 [untransfected]	0.018	0.023	0.034
U 937 [HIV A clone]	0.154	0.277	0.566
U937 [HIV A / Anti-A]	0.010	0.017	0.027
U 937 [HIV A/Anti-ABC]	0.013	0.021	0.035
U 937 [HIV A / Null DNA]	0.120	0.212	0.337

[B] Expression of Beta-galactosidase activity by *In situ* assay :

U 937 [untransfected] no blue spots in cells

U 937 [HIV A clone] blue spots in cells

U 937 [HIV A/Anti A] no blue spots in cells

U 937 [HIV A/Anti ABC] no blue spots in cells

U 937 [HIV A / Null DNA] blue spots in cells

Figure 51

Expression of Beta-galactosidase activity in extracts